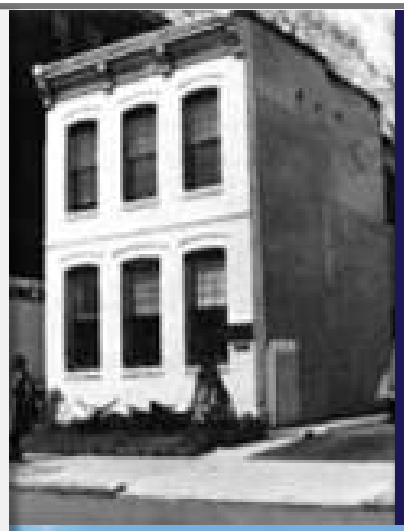
Phenotypic Characterization and Quality Control of Cells from Bacteria to Human Cells

Barry R. Bochner, Ph.D.
CEO & CSO, Biolog, Inc.
October 29, 2015

ATCC

About ATCC

- Founded in 1925, ATCC is a non-profit organization with headquarters in Manassas, VA
- World's premiere biological materials resource and standards development organization
- ATCC collaborates with and supports the scientific community with industry-standard biological products and innovative solutions
- Strong team of 400+ employees; over one-third with advanced degrees



Established partner to global researchers and scientists

Certification and Accreditation

ISO 9001:2008 Certification for quality management system

- Demonstrates commitment to quality products, customer service, and continued improvement

FM 516121

ISO 13485:2003 Certification for the design, development, production, testing, and distribution of medical devices

- Applies to synthetic molecular standards, the HIV surveillance kit, and other diagnostic and research kits

FM 610678

ISO Guide 34:2009 accreditation for production

- Applies to Certified Reference Materials (CRMs)

ISO/IEC 17025:2005 accreditation for testing

- Applies to all ATCC cultures, derivatives, and bioproducts tested in our laboratories

ATCC

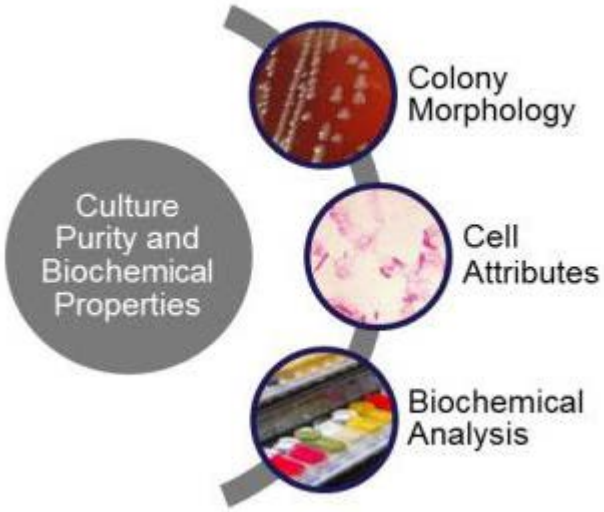
Highly Characterized Microbial Reference Strains

ATCC utilizes both classical and modern techniques

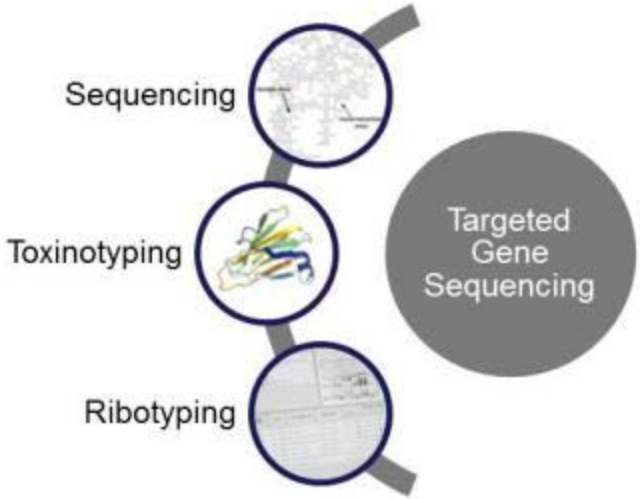
- Phenotypic analysis
- Genotypic analysis
- Proteotypic analysis
- Functional analysis

No single method of identification is sufficient

Phenotypic testing



Genotypic testing



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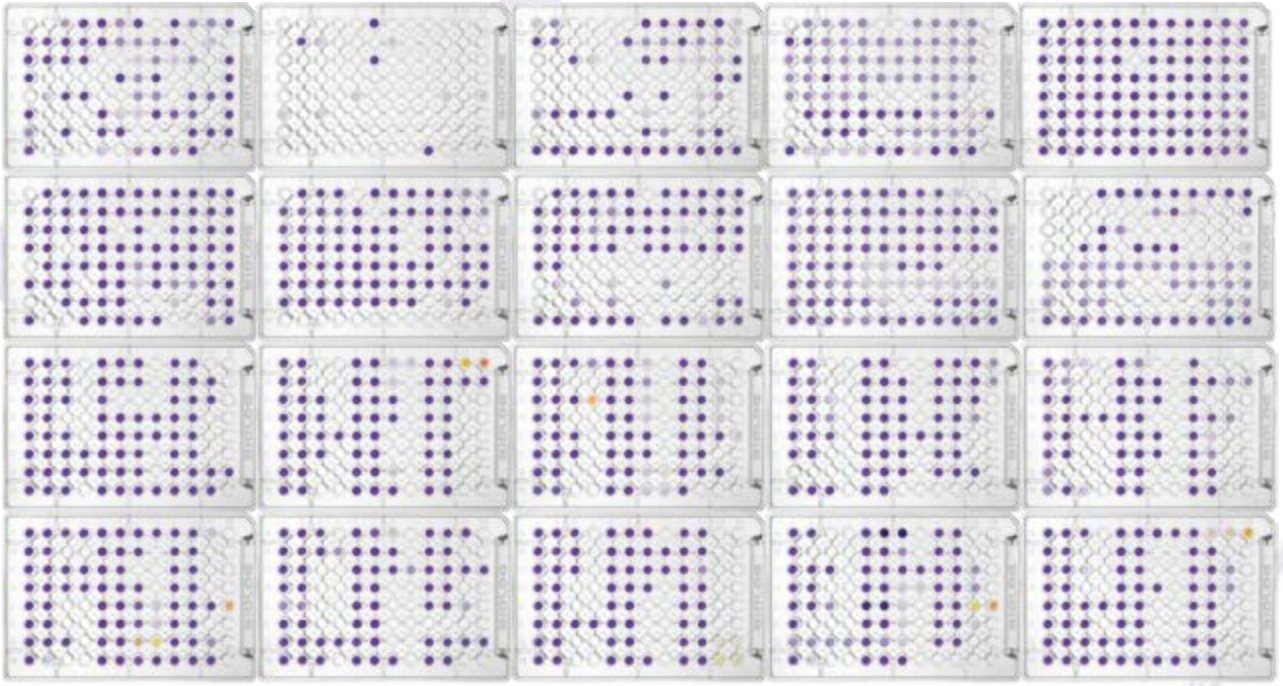
Phenotypic Characterization and QC of Cells

From Bacteria to Human Cells



Barry Bochner, Biolog, Inc., bbochner@biolog.com

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Part One:

Phenotypic Characterization of Cells

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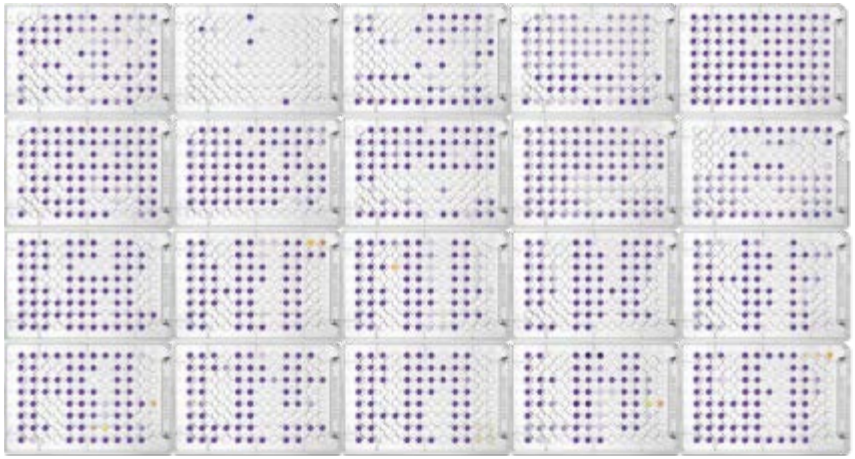
Tremendous Advances in Phenotyping Technology

1970s Technology

20 tests



21st Century Technology

2,000 tests

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Tremendous Advances in Phenotyping Technology

- Hundred fold expansion in tests
- Much broader range of tests
- Single dye, single color chemistry
- Bacteria
- Yeast
- Filamentous fungi
- Algae
- Human and other Animal Cells
- Kinetic phenotypes

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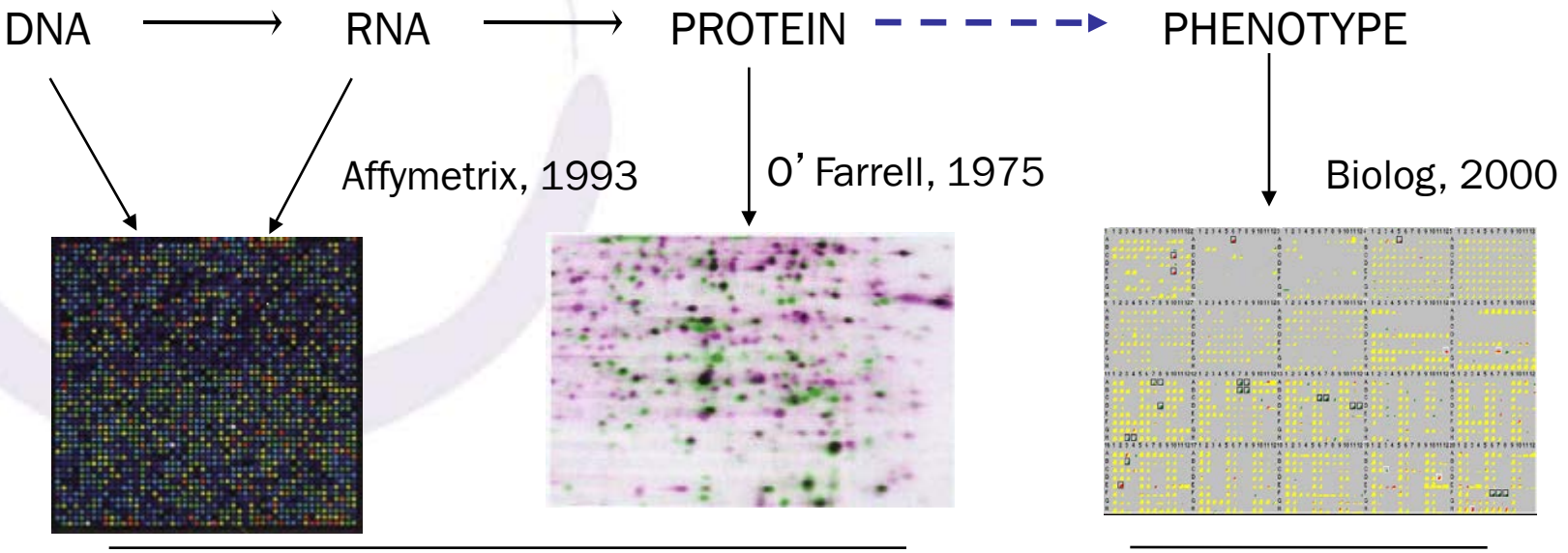
Phenomics = High Content Cell Phenotyping



Transcriptomics

Proteomics

Phenomics

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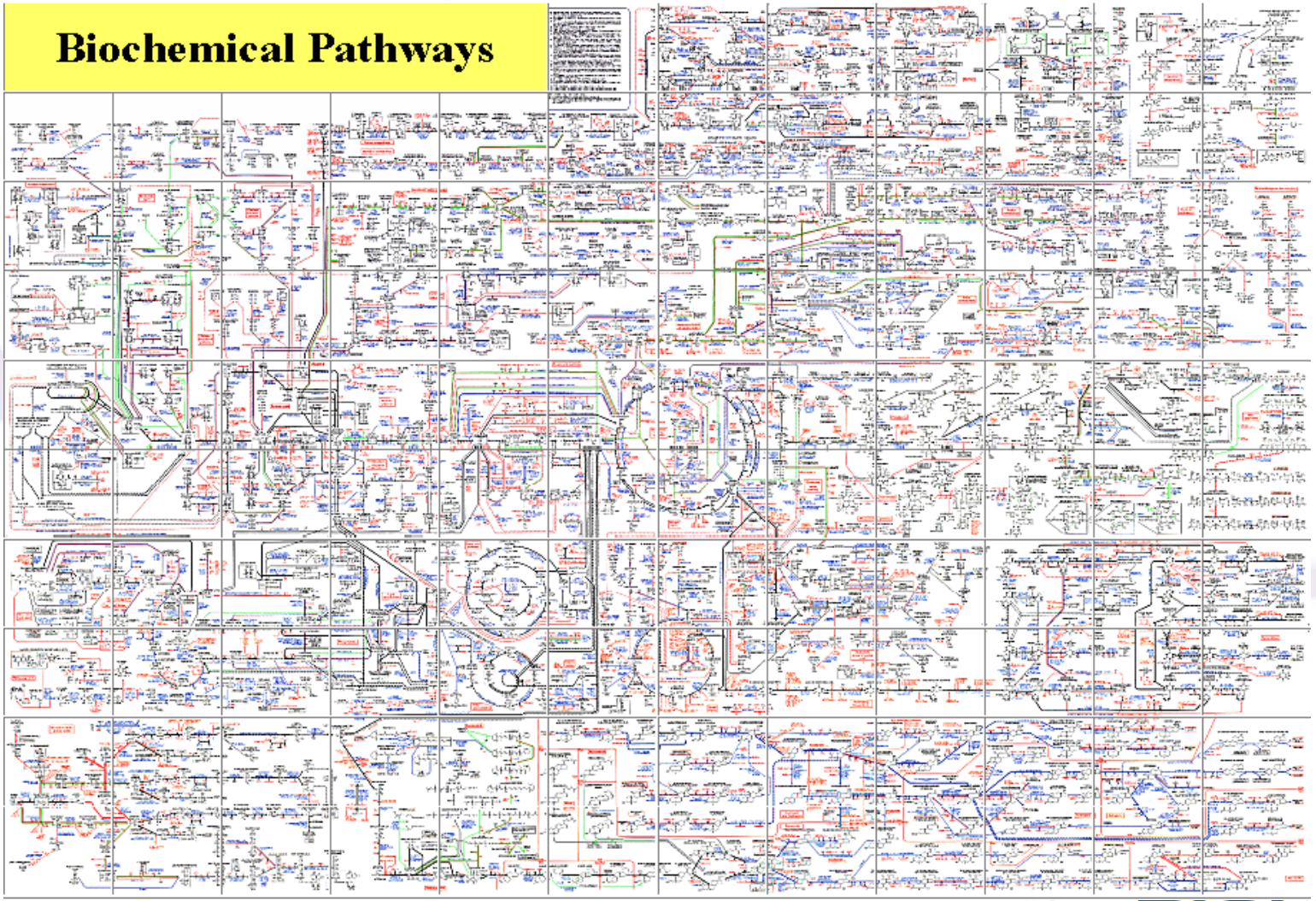
Complex Metabolic Circuitry of Cells



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Assay Principal:

Colorimetric Analysis of Energy Production

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Metabolism of C-sources Produces an Electron Flow

fructose

Testing sub-circuits within the cell's master circuitry

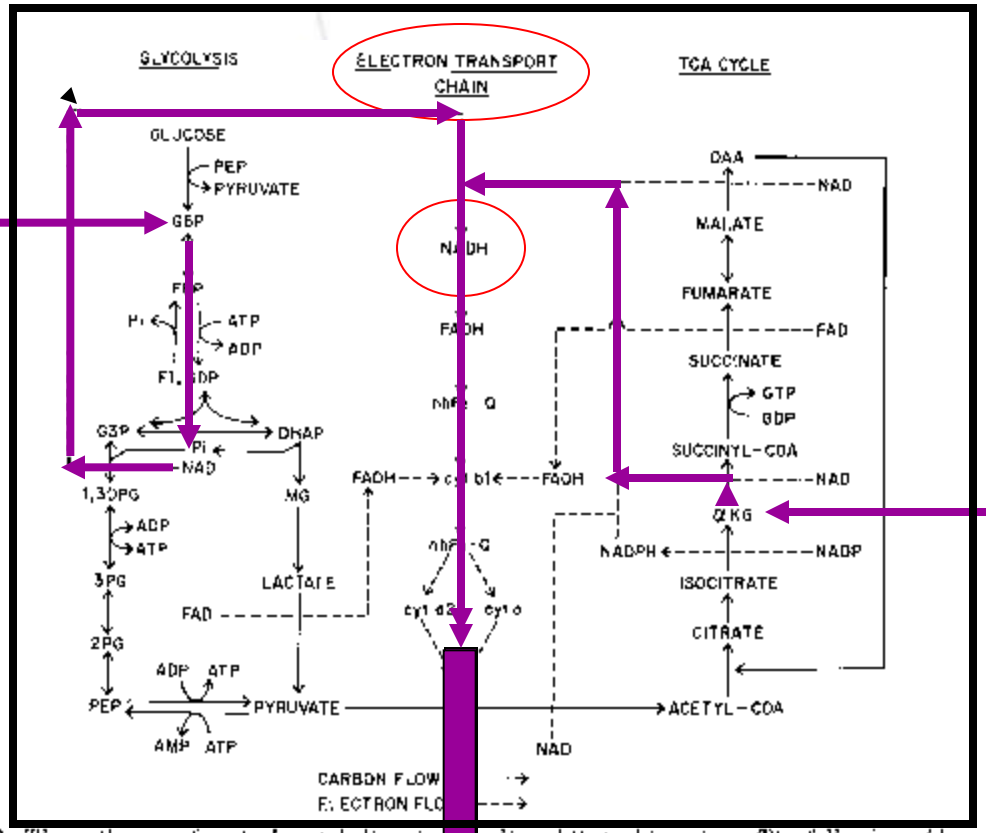

FIG. 3. *The pathways of central metabolism in* [illegible] *coli and S. typhimurium. The following abbreviations are used: glucose-6-P (G6P), fructose-6-P (F6P), f[illegible]tose-1,6-diP (F1,6DP), glyceraldehyde-3-P (G3P), 1,3-diP-glycerate (1,3DPG), 3-P-glycerate (3PG), 2-P-g[illegible]erate (2PG), P-enolpyruvate (PEP), dihydroxyacetone-P (DHAP), methyl glyoxal (MG), non-heme iron [illegible]yme Q complex (nhFe-Q), cytochrome (cyt), oxaloacetate (OAA), and α-ketoglutarate (αKG).*

glutamine

Redox Dye

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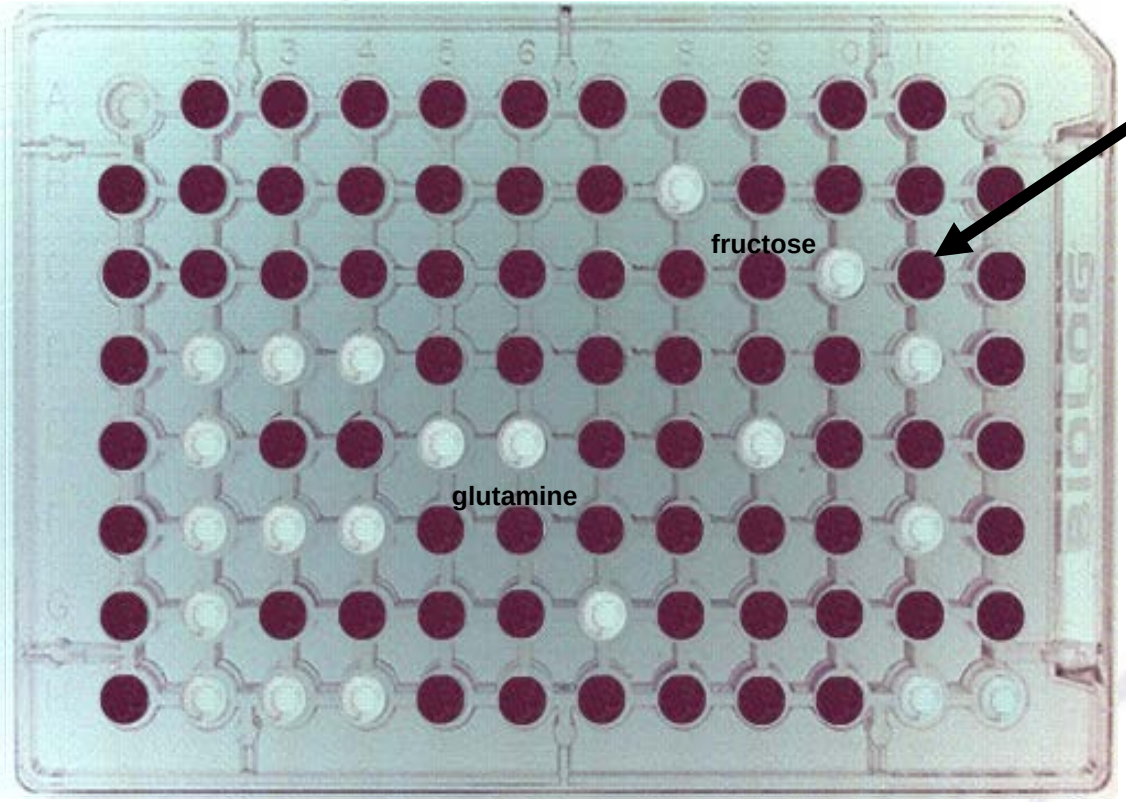
Redox Chemistry Measures Cell Energetics

Microplate containing a negative control well and 95 different carbon substrates



Stimulatory chemicals enhance energy production

Add cells → Add redox dye

Wells contain different tests and measure different pathway activities and phenotypes of cells

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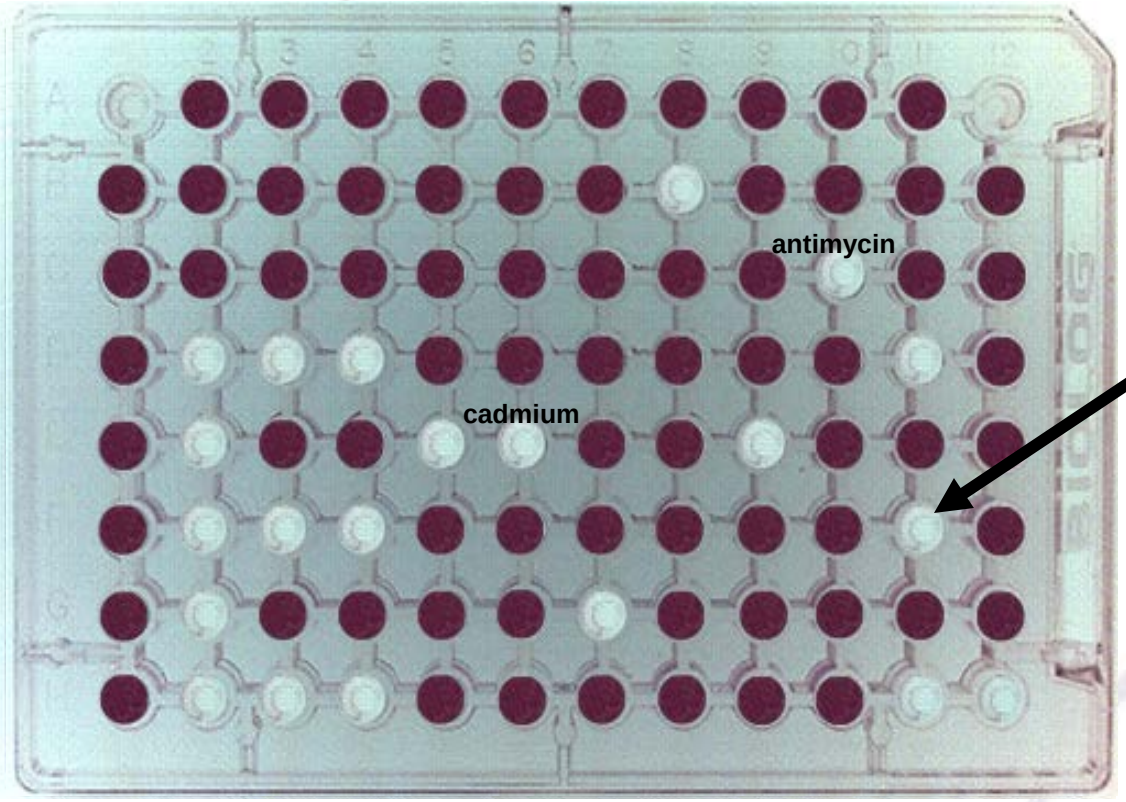
Redox Chemistry Measures Cell Energetics

Add cells → Add redox dye



Inhibitory chemicals block energy production

Wells contain different tests and measure different pathway activities and phenotypes of cells

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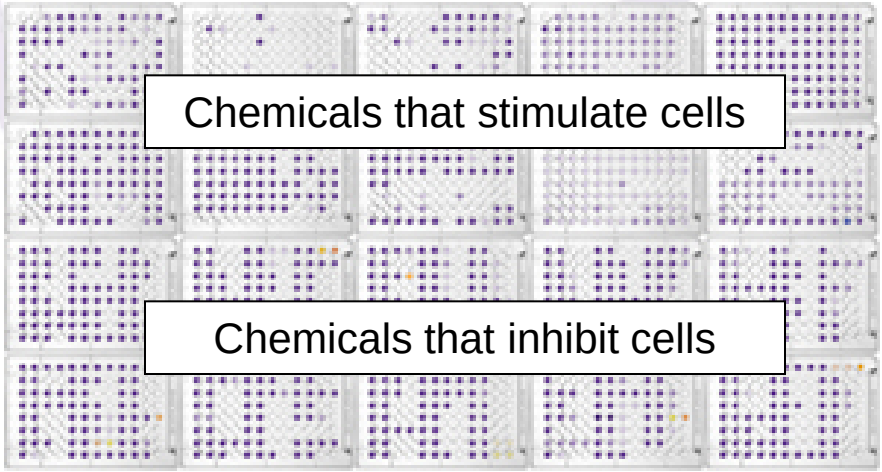
2 Components of the Phenotyping Assay Platform

Phenotype MicroArrays™



colorimetric cell assays in 96-well microplates

OmniLog™ Incubator/Reader

incubation and recording of data in the OmniLog

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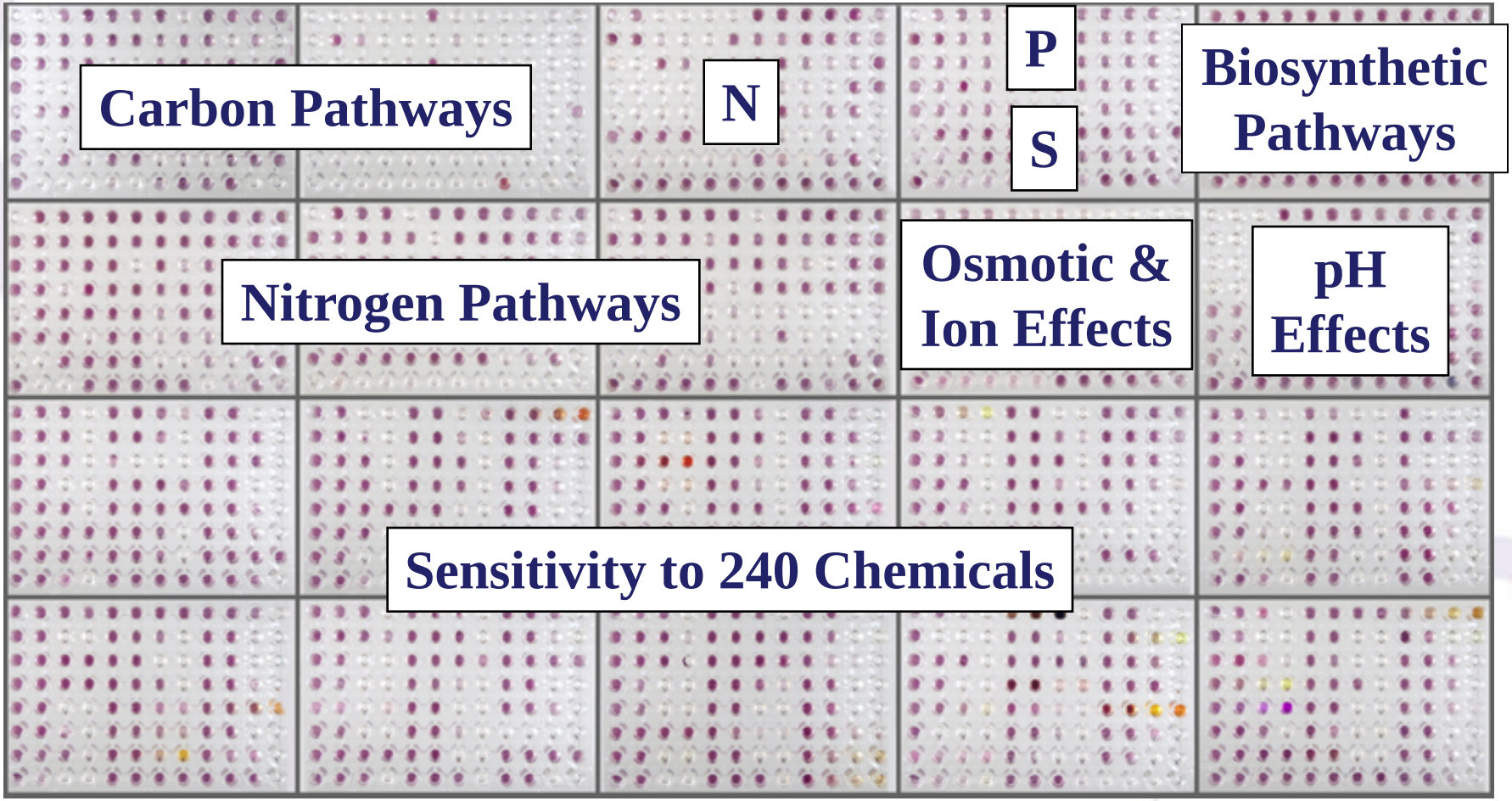
PM Platform - ~2,000 Phenotypic Assays



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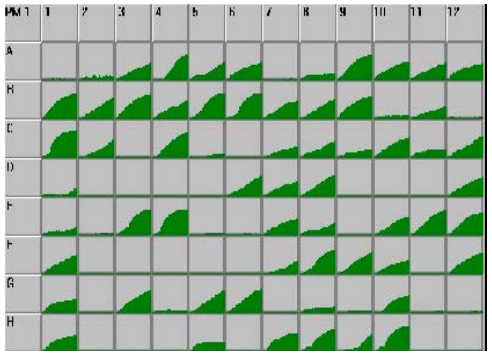
PM Assays are Easy to Run

Assays Initiated by adding cells to wells

100 µl per well

OmniLog PM System

Holds 50 microplates at a set temperature and measures color formation at 15-minute intervals



Kinetic assay readout for up to 5,000 wells

CVs typically < 10%

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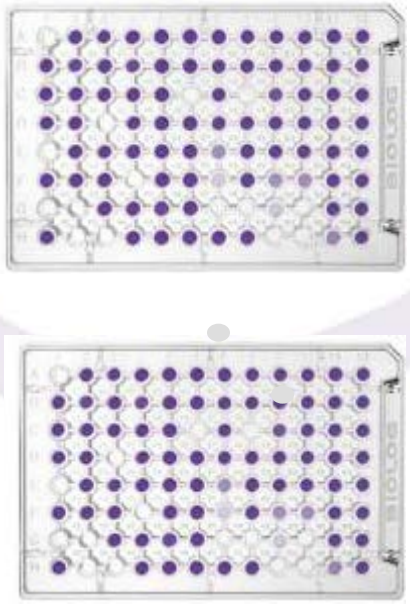
PM Platform - Comparing Two Cell Lines

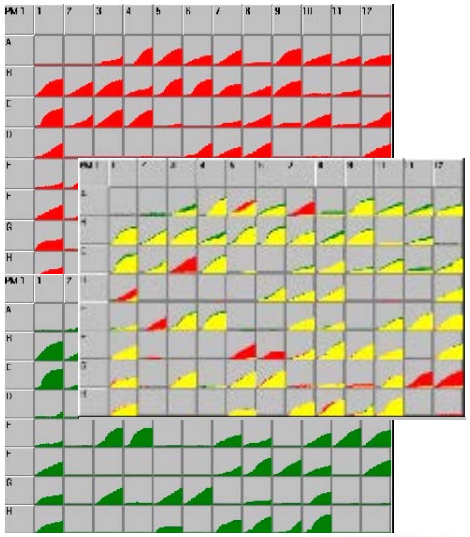
Add cell A

Add cell B



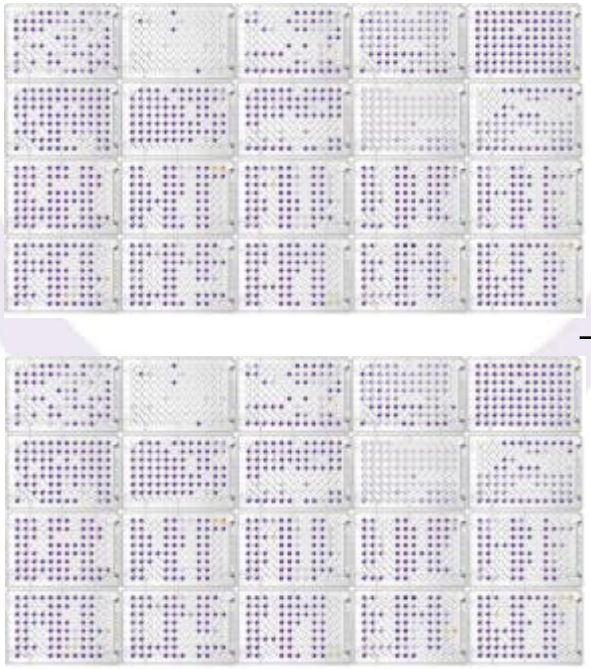
PM Pattern

OmniLog PM System



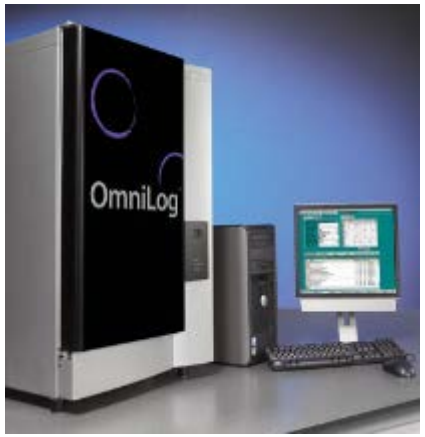
PM Kinetic Result

PM Platform – Comparing Two Cell Lines



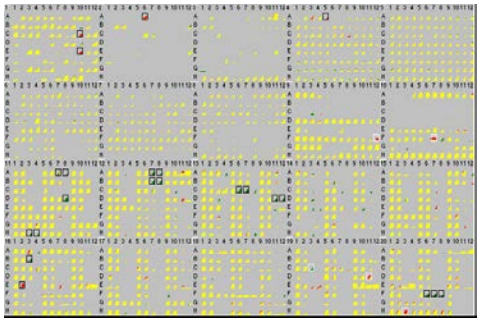
PM Pattern

1 hr



OmniLog PM System

Automatic



PM Kinetic Result

24-48 hr

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Examples of Phenomic Applications

- Profiling metabolism and chemical sensitivities of a cell
- Comparing properties of pathogenic vs non-pathogenic strains
- Determining the function(s) of a gene
- Analyzing environmental effects on cell phenotypes
- Optimizing production of a cell product – e.g. a toxin

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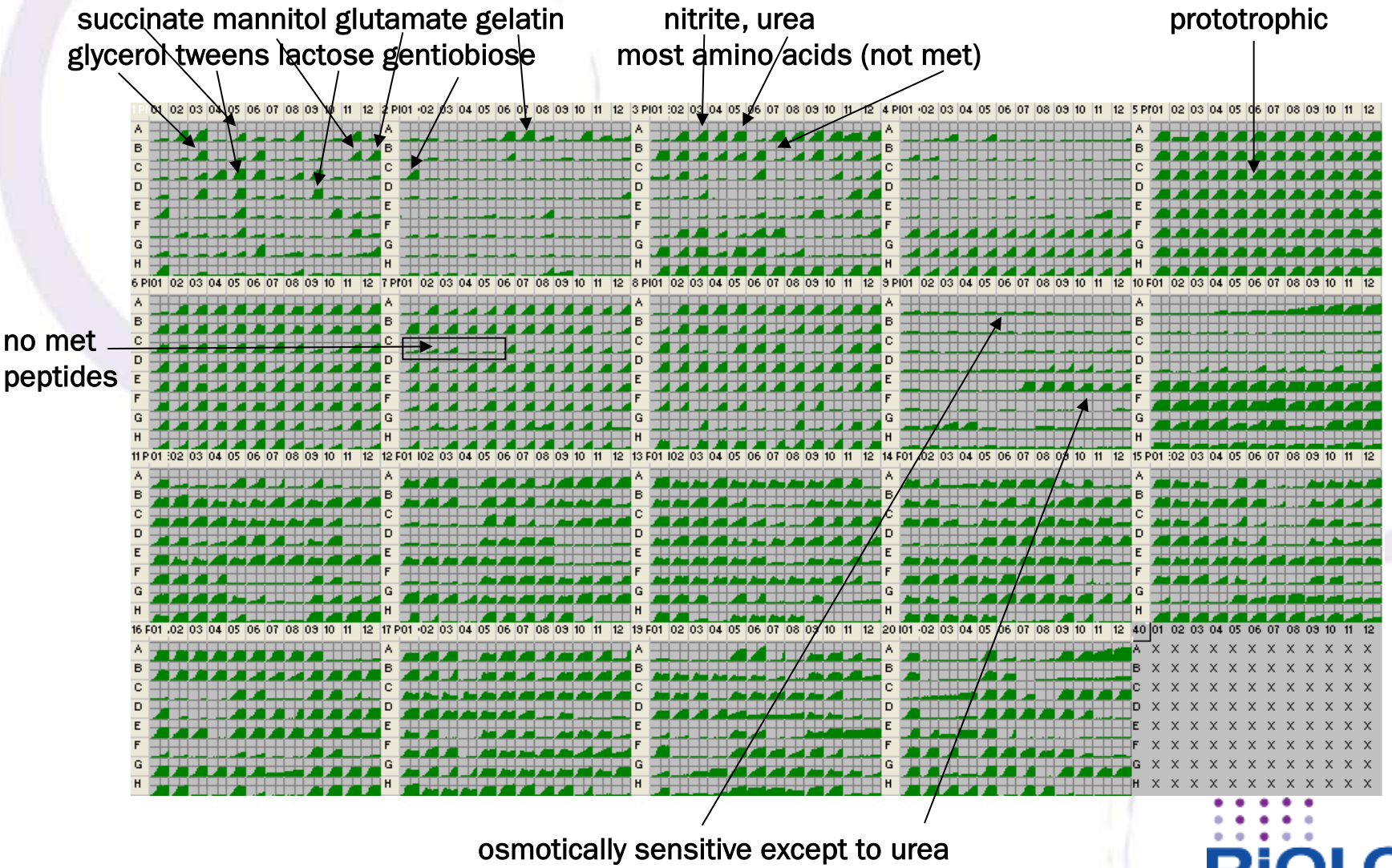
PM Analysis of Streptomyces coelicolor



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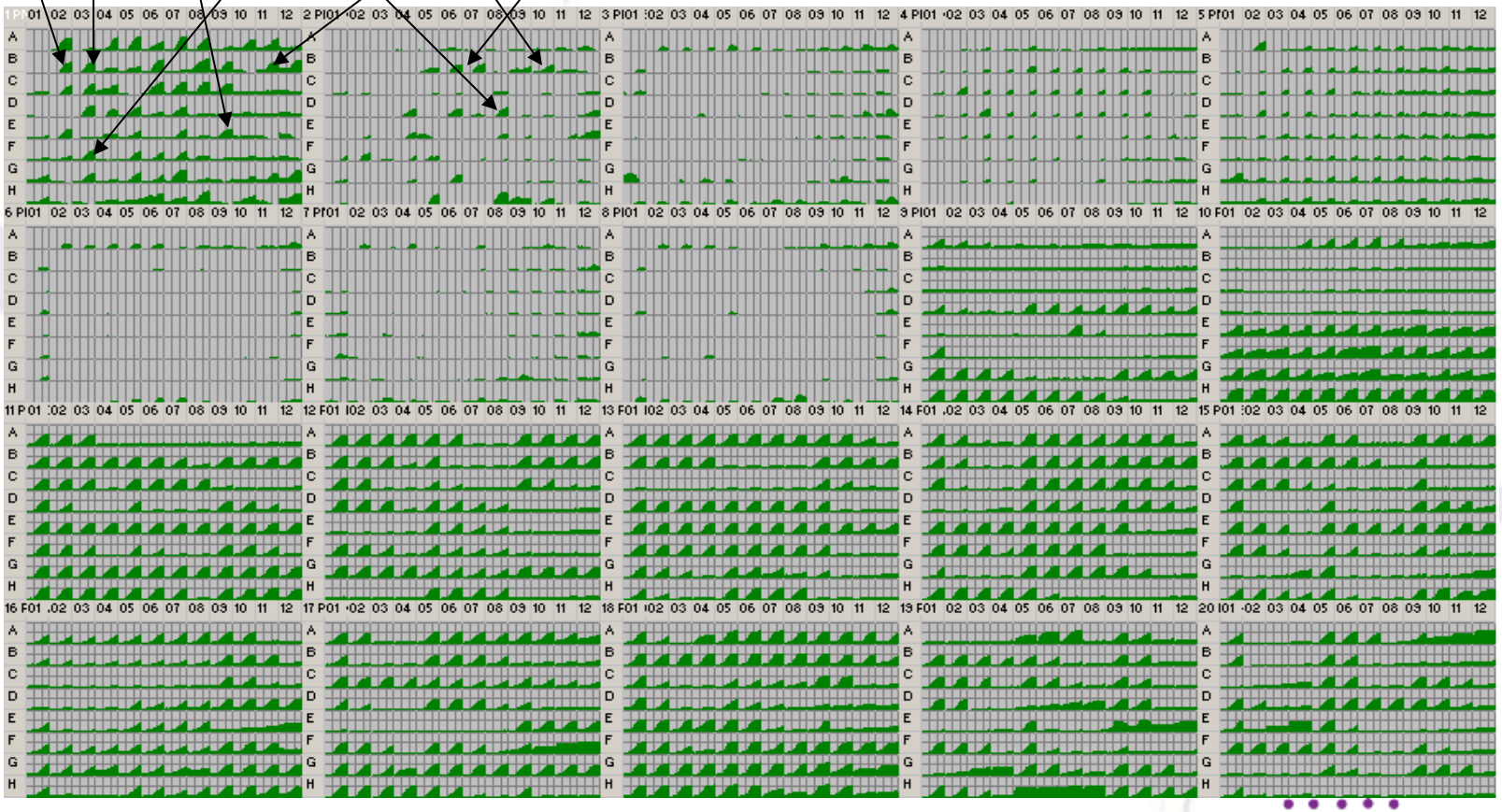
PM Analysis of Mycobacterium smegmatis MC^2-155

sorbitol, adonitol, xylitol, erythritol

glycerol, inositol, mannitol, arabitol

Also – glucose, trehalose, glutamate, serine, alanine

gluconate, acetate, acetoacetate, tween40 and others



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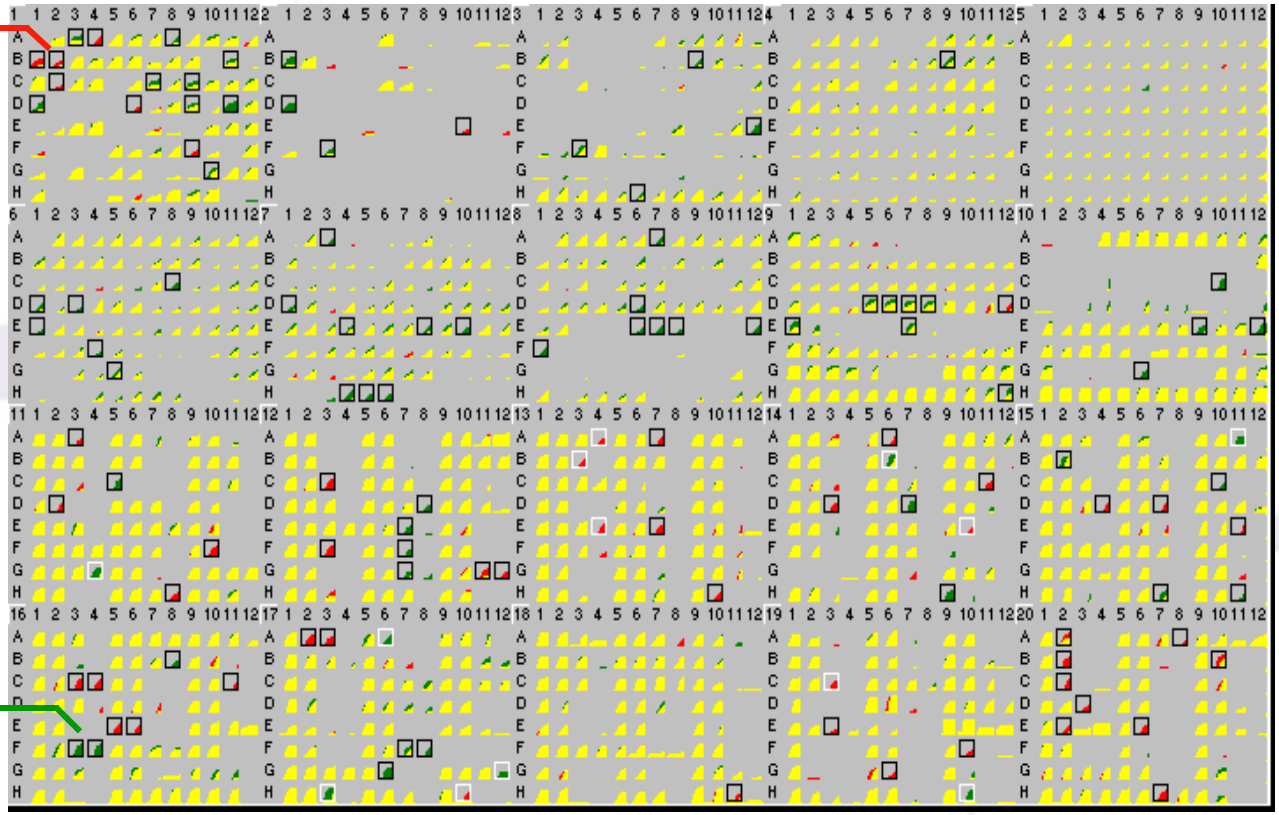
Comparing Two E. coli Strains:

Pathogenic (0157) vs non-Pathogenic (MG1655) E. coli

sorbitol negative



tellurite resistant

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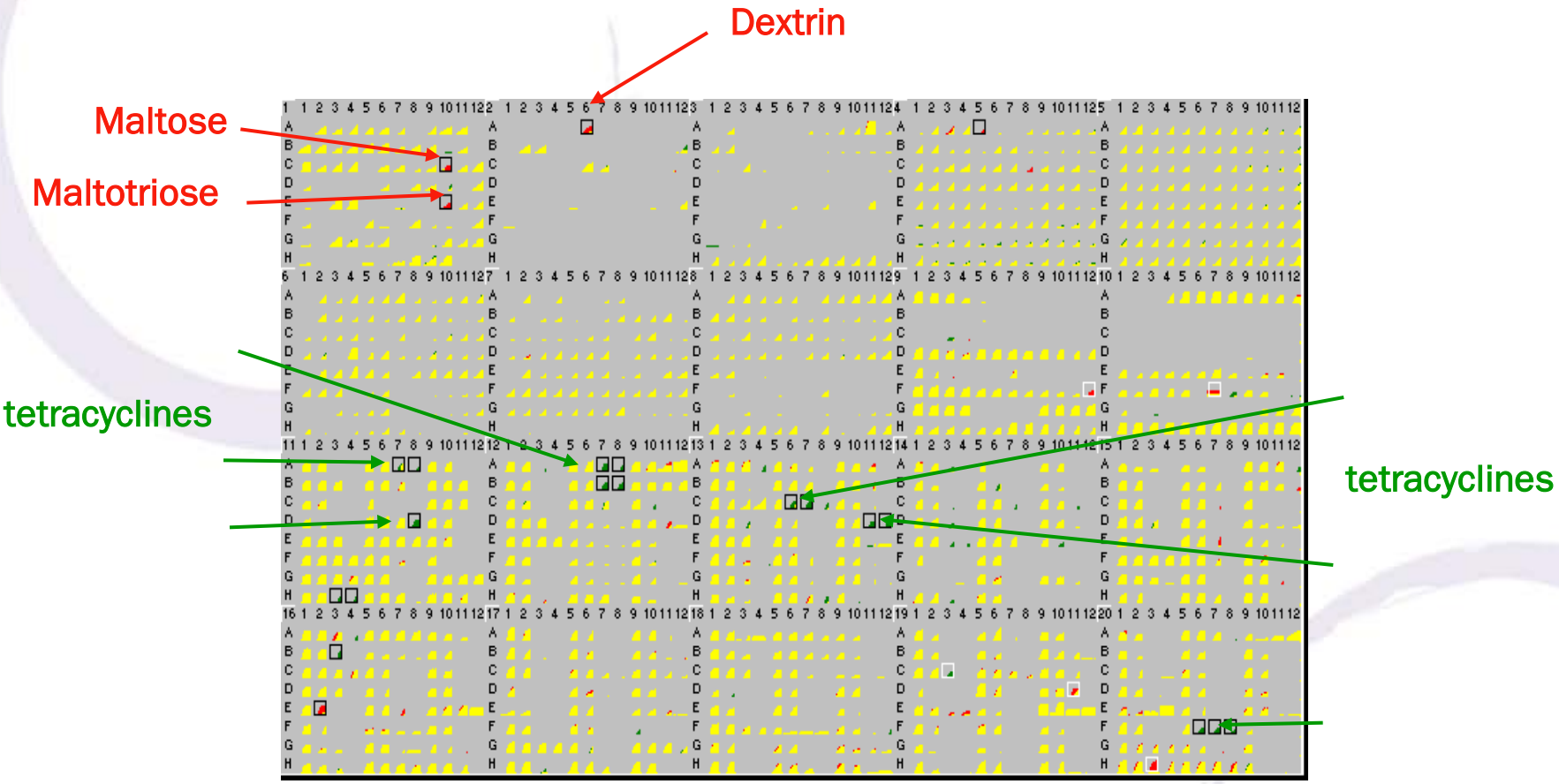
Comparing Wild Type vs Mutant:

E. coli malF::Tn10 vs MG1655



Red = Phenotypes Lost

Green = Phenotypes Gained

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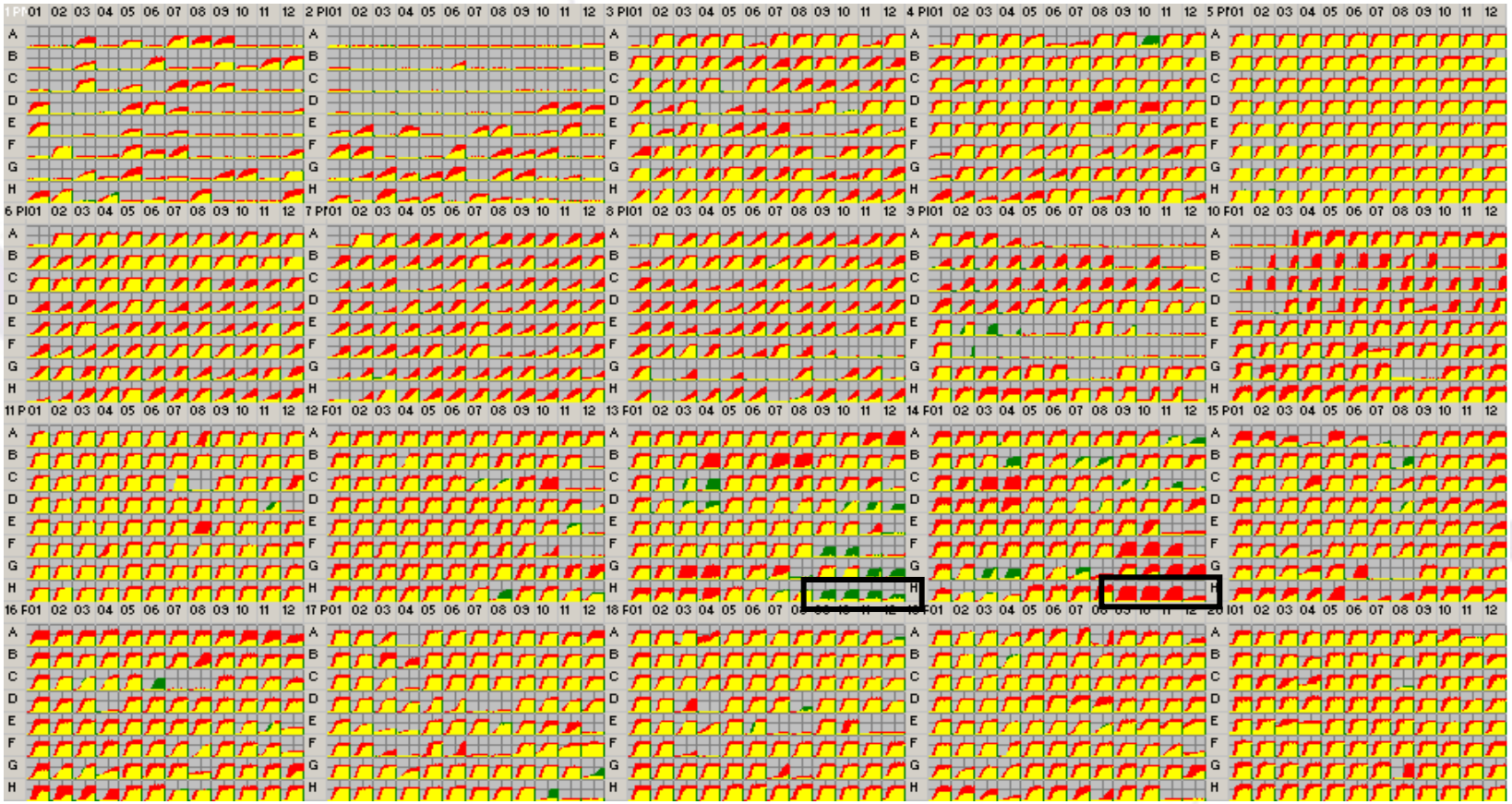
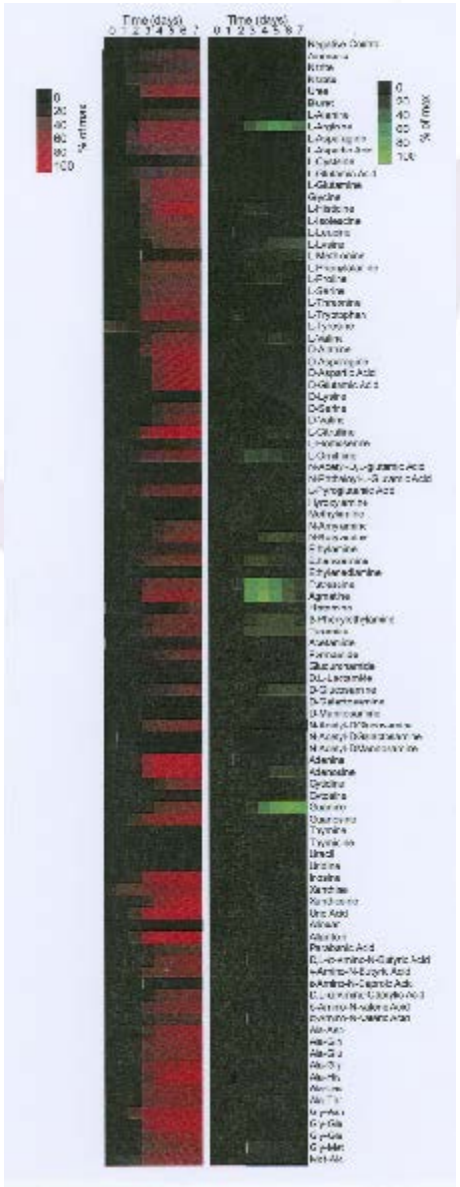
Comparing Psd.aeruginosa 8512 at 26 vs 36 C.



Culture Conditions Inducing Toxin Synthesis



Culture conditions inducing synthesis of a trichothecene mycotoxin in the wheat pathogen, *Fusarium graminearum*. A special strain was constructed with a toxin gene promoter fused to GFP.

Induction was highest with arginine, putrescine, agmatine, and guanine as nitrogen sources.

D. Gardiner et al – Fungal Gen & Biol (2009)

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Phenotyping Human Cells:

Analyzing Human Cell Metabolism, Human Genes, Human Disorders, and Effect of Drugs

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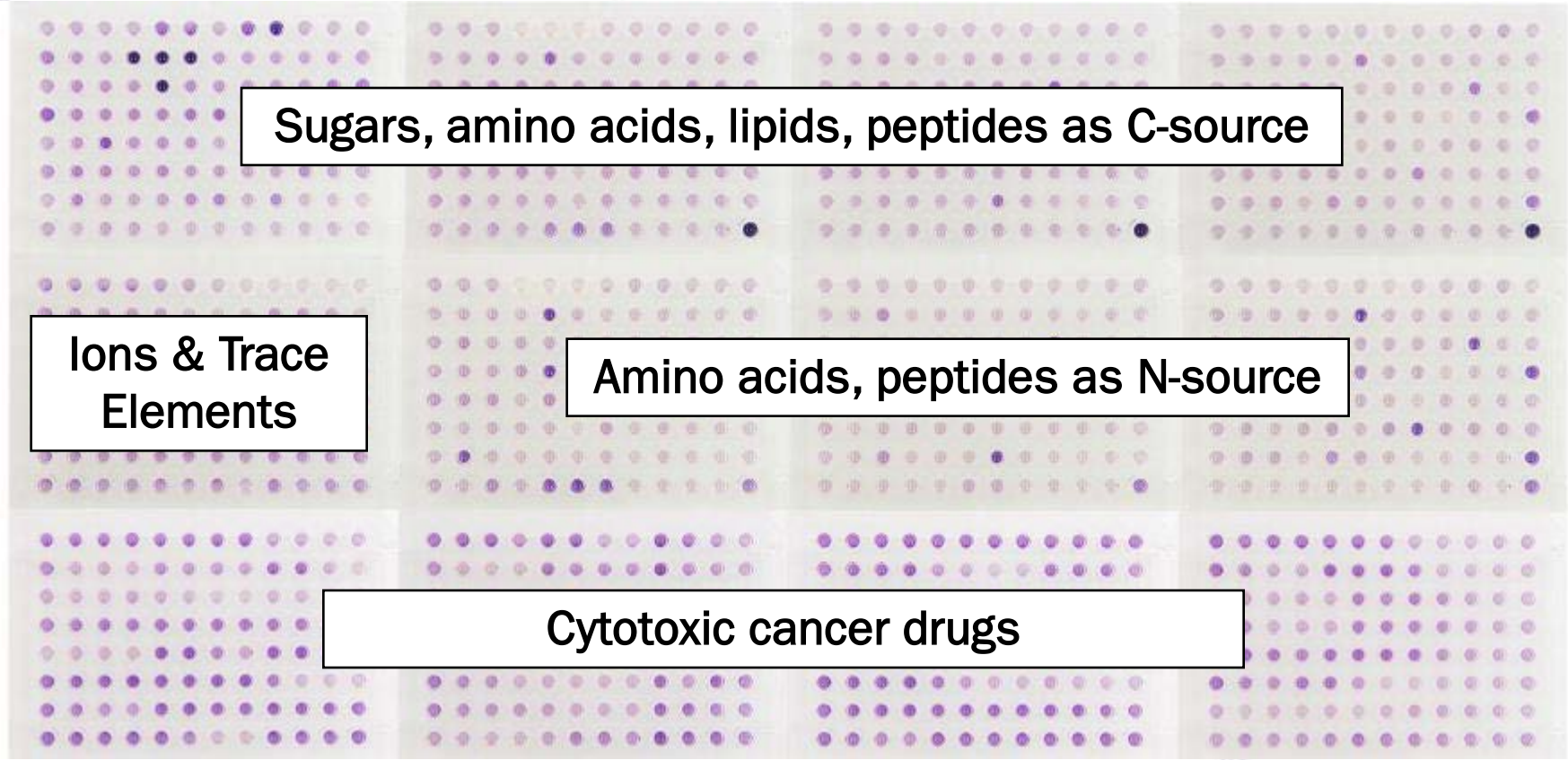
~1500 Assays and Culture Media for Mammalian Cells



Plus 3 panels with hormones, cytokines and other bioactives

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Harry Eagle, JBC 1958

The Utilization of Carbohydrates by Human Cell Cultures

HARRY EAGLE, STANLEY BARBAN, MINA LEVY, AND HENRY O. SCHULZE

From the Section on Experimental Therapeutics, Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Public Health Service, United States Department of Health, Education, and Welfare, Bethesda, Maryland

(Received for publication, April 21, 1958)

TABLE I

Ability of a variety of carbohydrates and related compounds to substitute for D-glucose in cell cultures*

The compounds were tested at varying levels (0.5 to 50 mM) in a glucose-free but otherwise "complete" growth medium supplemented with dialyzed serum. The letters in parentheses after each compound indicate the cell lines against which it was tested.

Compounds which did not support growth	Compounds permitting slight growth, but less than with glucose	Compounds permitting growth at rate comparable with that on glucose
	+/-	+
Lactose (U, H, L, T)	Cellobiose (U, G, H, K, M, T)	Trehalose (U, G, H, K, M, T)
Sucrose (H, L, T)	Melibiose (U, G, H, K, M, T)	Turanose (U, G, H, K, M, T)
D-Allose (G, T)	Sorbitol (all)	
D-Alrose (G, T)		D-Fructose (all)
D-Galactose (L)†	D-Talose (G, T)	D-Galactose (U, G, H, K, M, T)†
D-Galacturonic acid (K, T)	D-Xylose (all)	D-Glucose (all)
D-Gluconic acid (L, T)	D-Ribose (G, H, K)‖	D-Glucose 1-PO4 (H, K, M)
Methyl-α-D glucoside		D-Glucose-6-PO4 (H, K, M)
Methyl-β-D-glucoside		D-Mannose (all)
D-Glucuronic acid (K, T)		
D-Gulose (K, T)		
D-Mannitol (all)		
L-Rhamnose (K, T)		
D-Sorbose (all)		
L-Arabitol (G)	6	8
D-Arabinose (G)		
D-Lyxose (G)		
D-Ribose (G, L, M, T)‖		
Xylitol (G)		
D-Erythrose (H, L)		
Acetate (H, L, T)		
Citrate (H, L, T)		
Fumarate (H, L, T)		
Glycerol (H, L, T)		
Lactate (H, L)		
Malate (H, L, T)		
Phosphoglycerate (H, K)		
Pyruvate (all)		
Succinate (H, L, T)		

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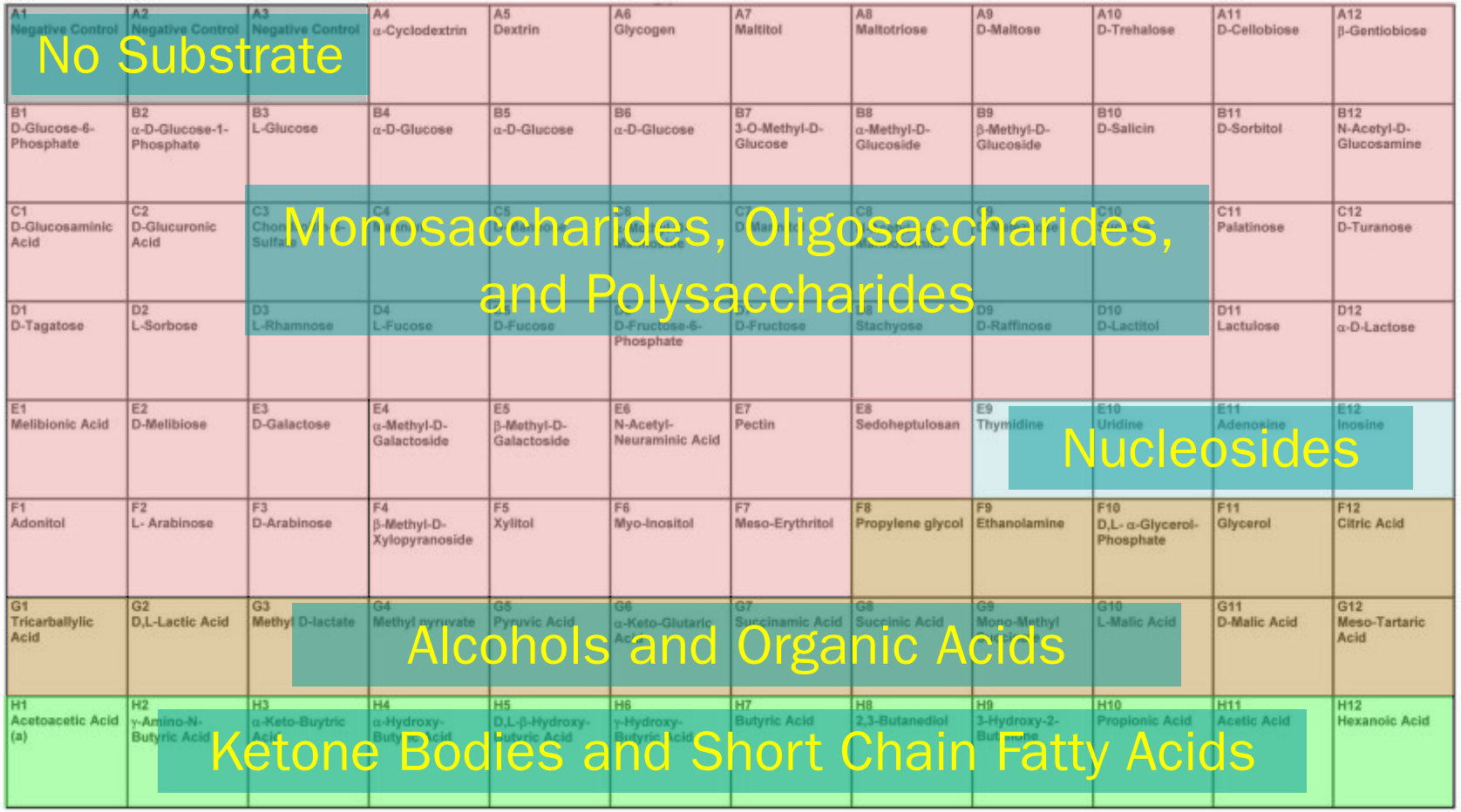
PM-M1 to M4: 367 Carbon-Energy Substrates for Cells



Each well has a different substrate

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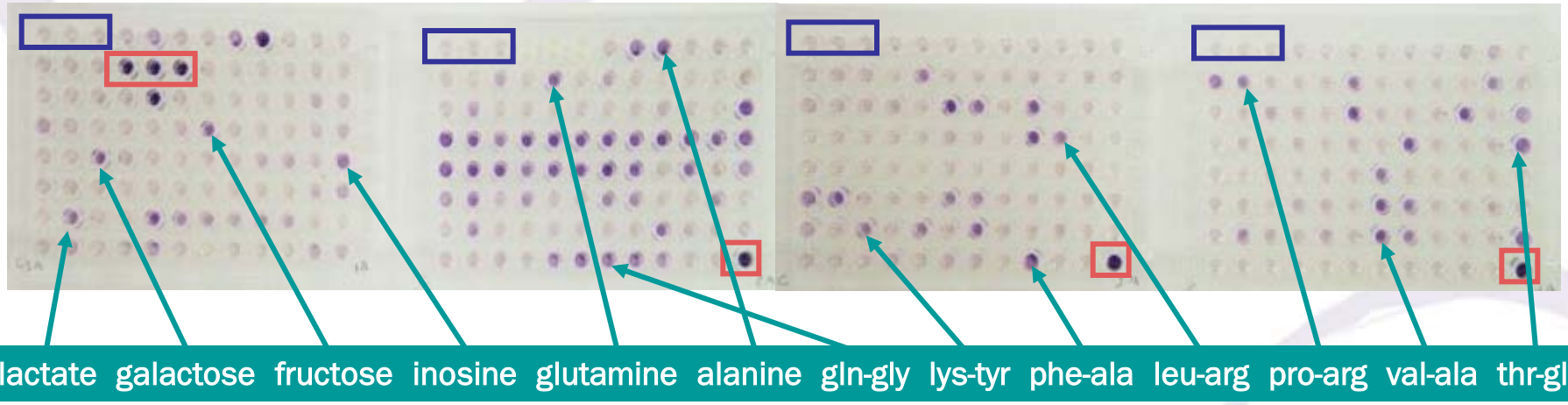
PMs Assay Carbon/Energy Pathways in Cells

Negative Controls

Positive Controls - Glucose

PM-M1 PM-M2 PM-M3 PM-M4



sugars, alcohols, acids

fatty acids, amino acids, and dipeptides

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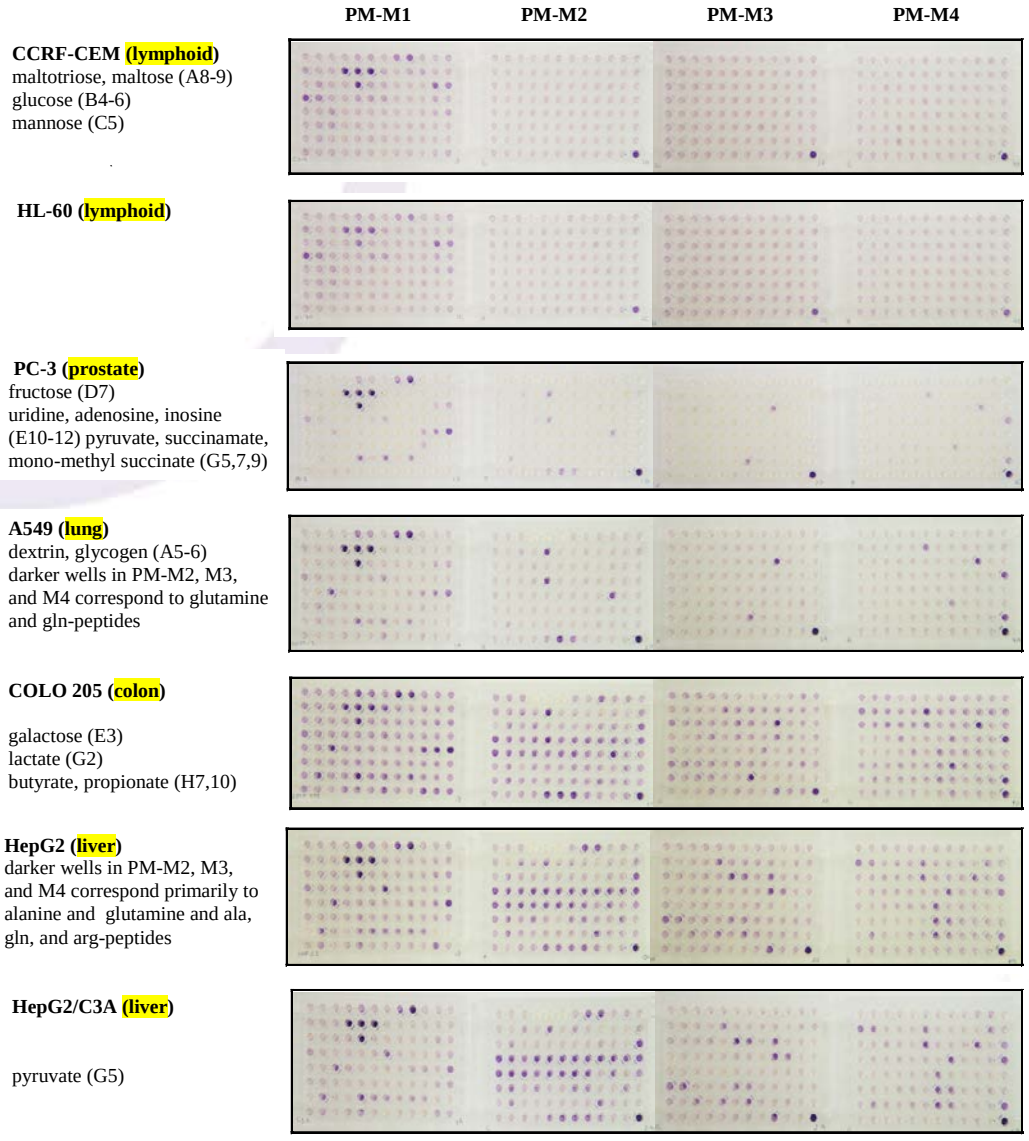
Each Cell has a Different Set of Energy Pathways



Adapted from Bochner et. al. PLoS ONE (2011) 6:e18147

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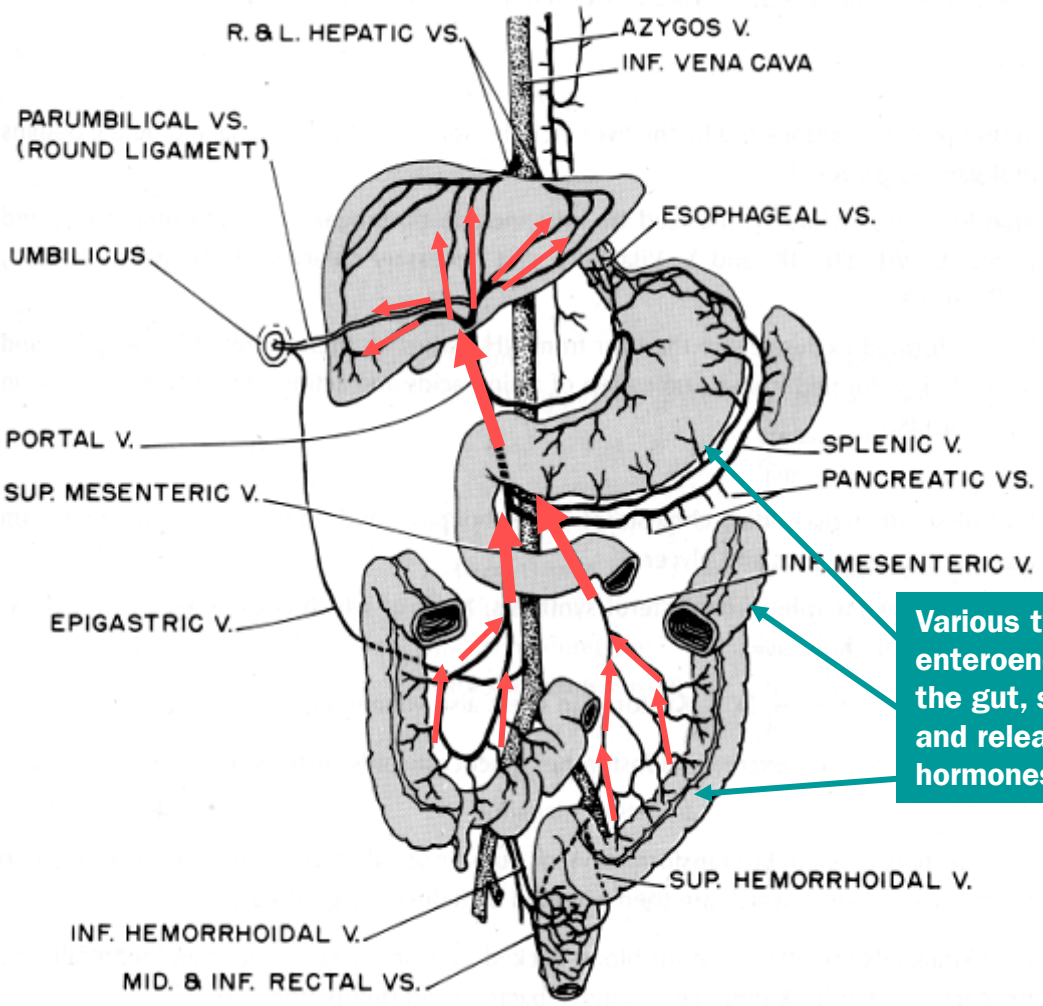
Physiology of Nutrient Absorption



Various types of enteroendocrine cells line the gut, sense nutrients, and release signaling hormones called incretins

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Analyzing Mutations in Human Cells

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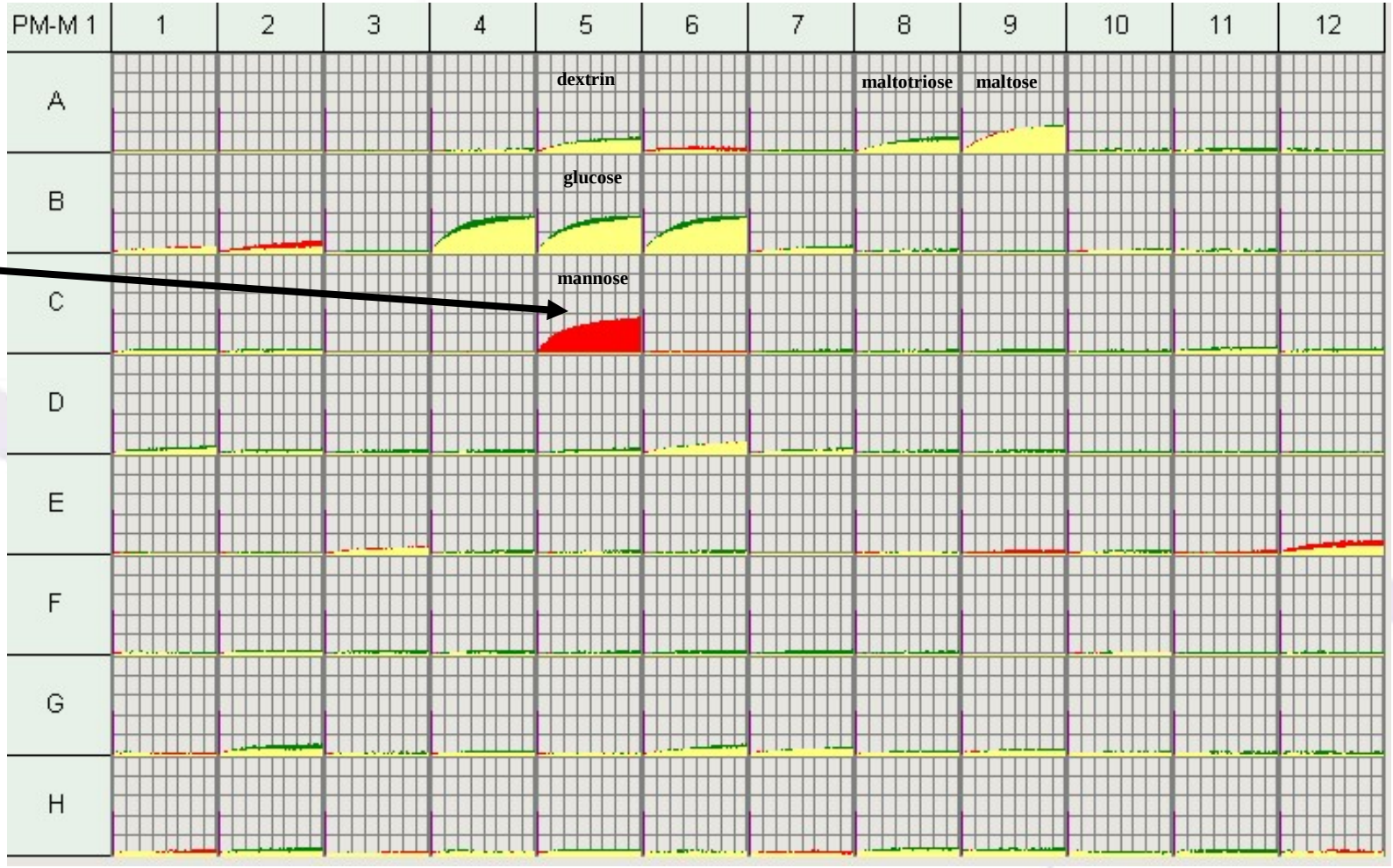
Comparison of HAP1 vs HAP1_MPI_124-16 on Biolog Redox Dye MB reduction (Average of 4) after dispensed into PM-M1 plates at 20,000/well in IF-M1 + 0.3 mM Gln + 5% FCS + PS medium and incubated for 20 h.

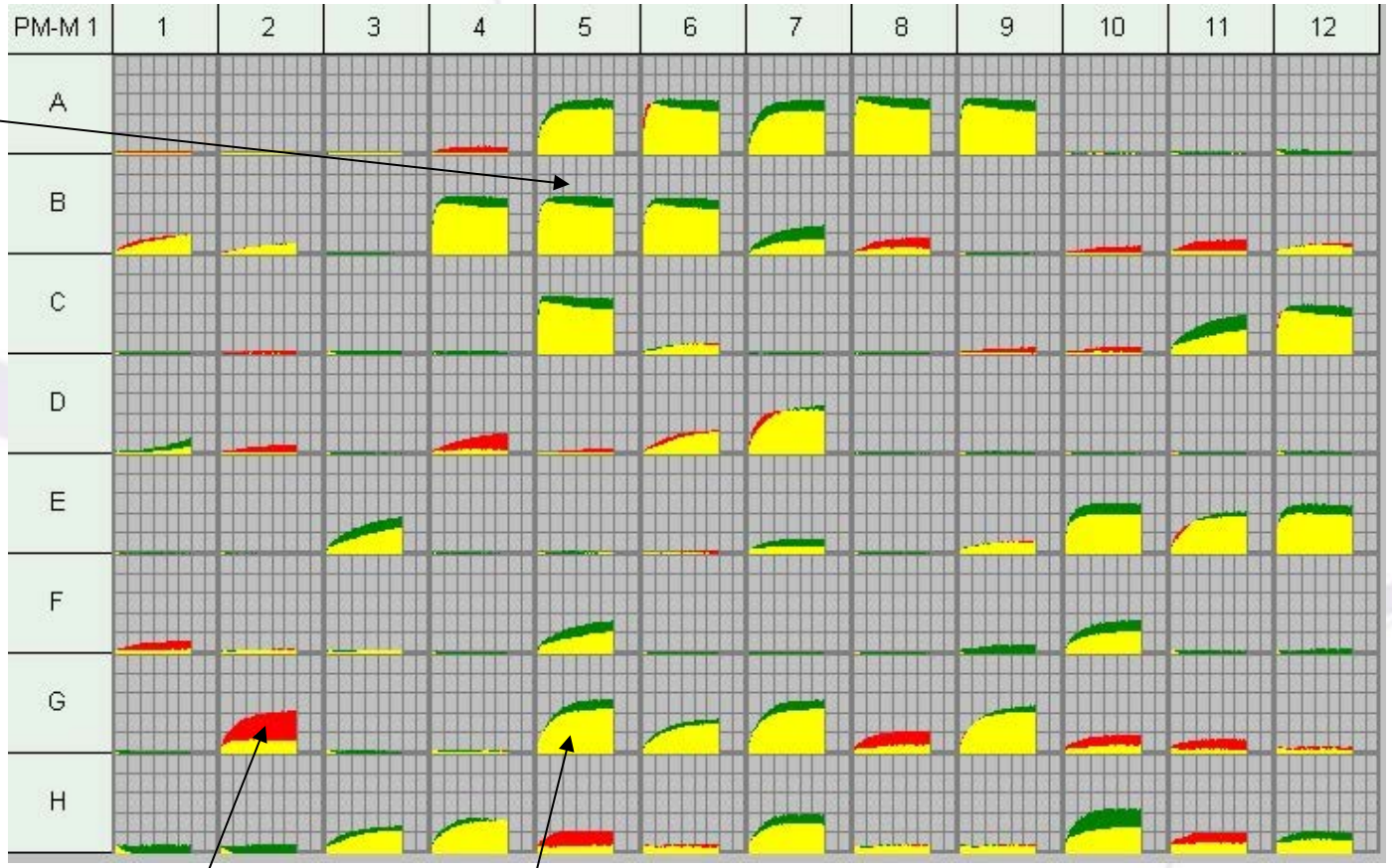
Mannose phosphate isomerase mutant loses metabolism of Mannose



HAP1 and HAP1_MPI_MPI_124-16 (Mannose Phosphate Isomerase) , both grown in IMDM + 10% FCS + PS, were dispensed into a PM-M1 plate at 20,000 cells/well in 50 uL IF-M1 + 0.3 mM Gln + 5% FCS + PS, incubated 20 h at 37°C under 5%CO_2-95% air before adding 10 uL/well Dye MB, plates sealed with tape and placed in a 37°C OmniLog. Dye reduction over ten hour minus background

Metabolic Changes in MCF10a vs PI3K Clone CL1

Red = Normal Cell, Green = Cancer Cell



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Metabolic Changes in MCF10a vs PI3K Clone CL1

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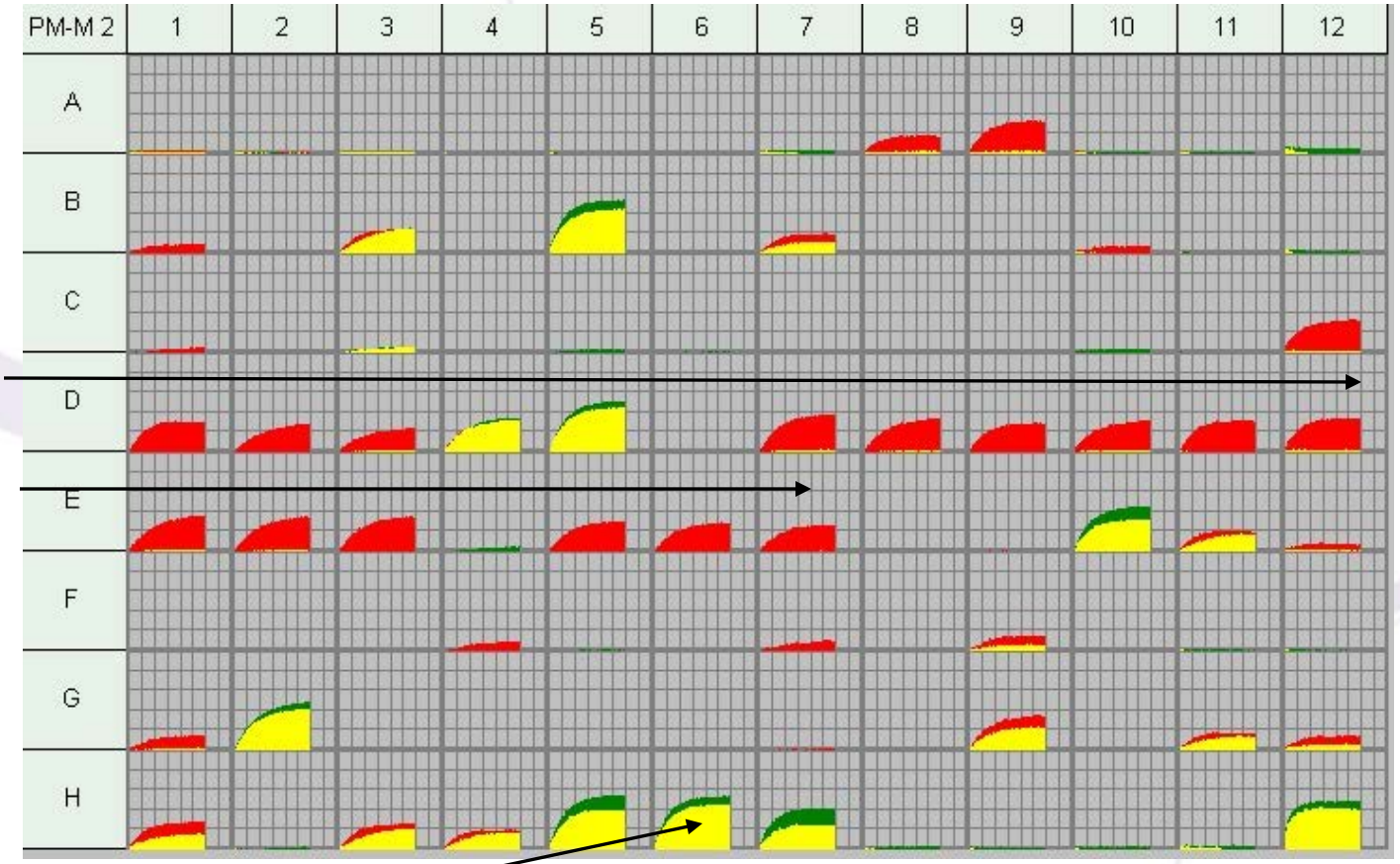

Alanine peptides

Glutamine peptides

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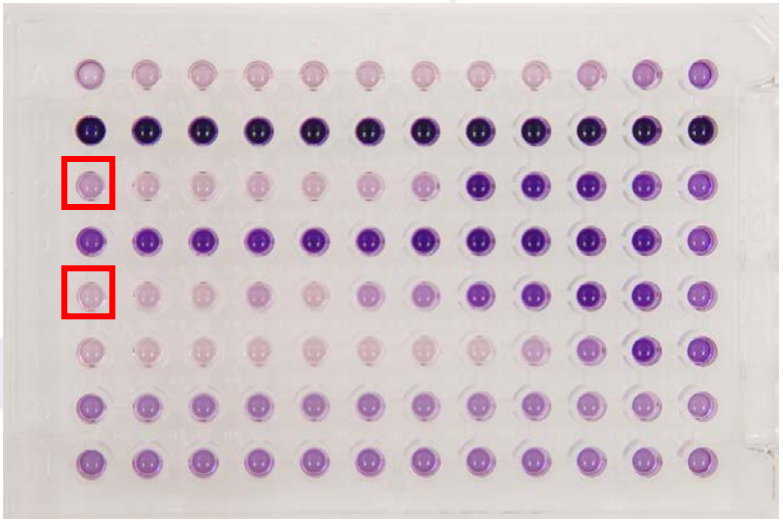
Assay medium: IF-M1 +5% horse serum +0.3mM Gln +1xP/S+ 0.1 ug/mL cholera toxin +10 ug/mL insulin+0.5 ug/mL hydrocortisone +0.2 ng/m EGF

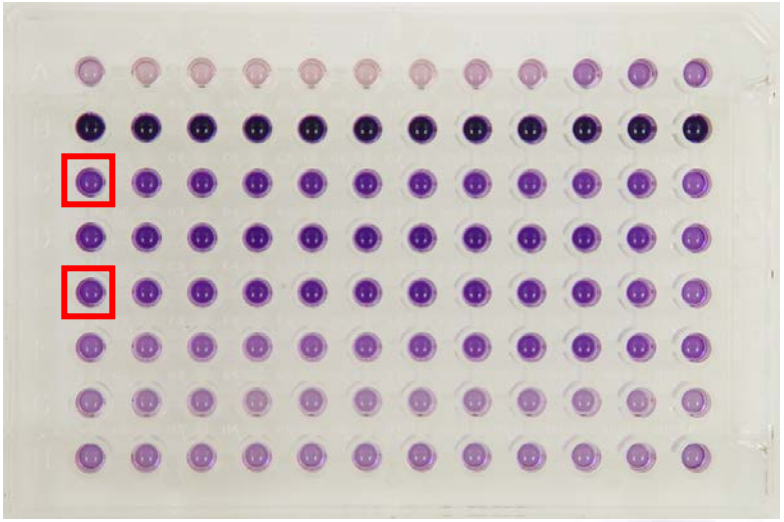
Cells: 20k/well

20141208-12_PM-M Tox2_PI3K Inhibitor Titration_68h in CO_2_24h in OL with Dye MA

MCF10a / PIK3CA (H1047R/+)



MCF10a (WT)



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Part Two:

Quality Control of Cells

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Who Needs to Perform Cell Line QC ?

- Culture collections and cell banks
- Bioprocess scientists banking cells (seed cultures) to inoculate fermentations
- Cell-based assay labs
- Anyone using cells in their research

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How is Cell Line QC Currently Performed ?

For Microbial Cells

- A mixture of genetic and phenotypic tests

For Human and Animal Cells

- STR profiling according to cell line authentication method ASN-0002 (genetic method)
- Many labs rely on judging cell morphology

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Analyzing Metabolic and Other Phenotypes Provides a Complementary QC Method

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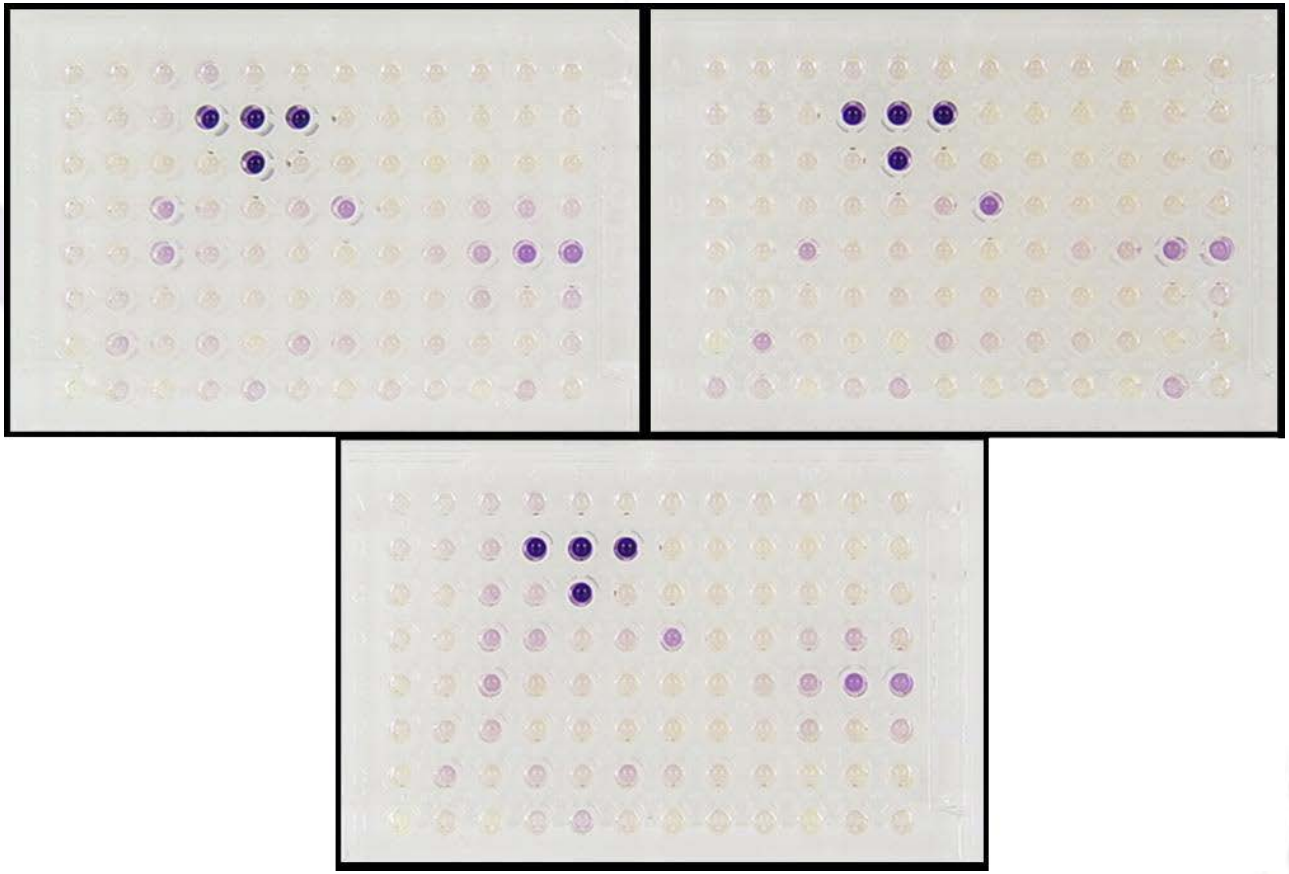
PM Fingerprints are Highly Reproducible



CVs typically are less than 10%

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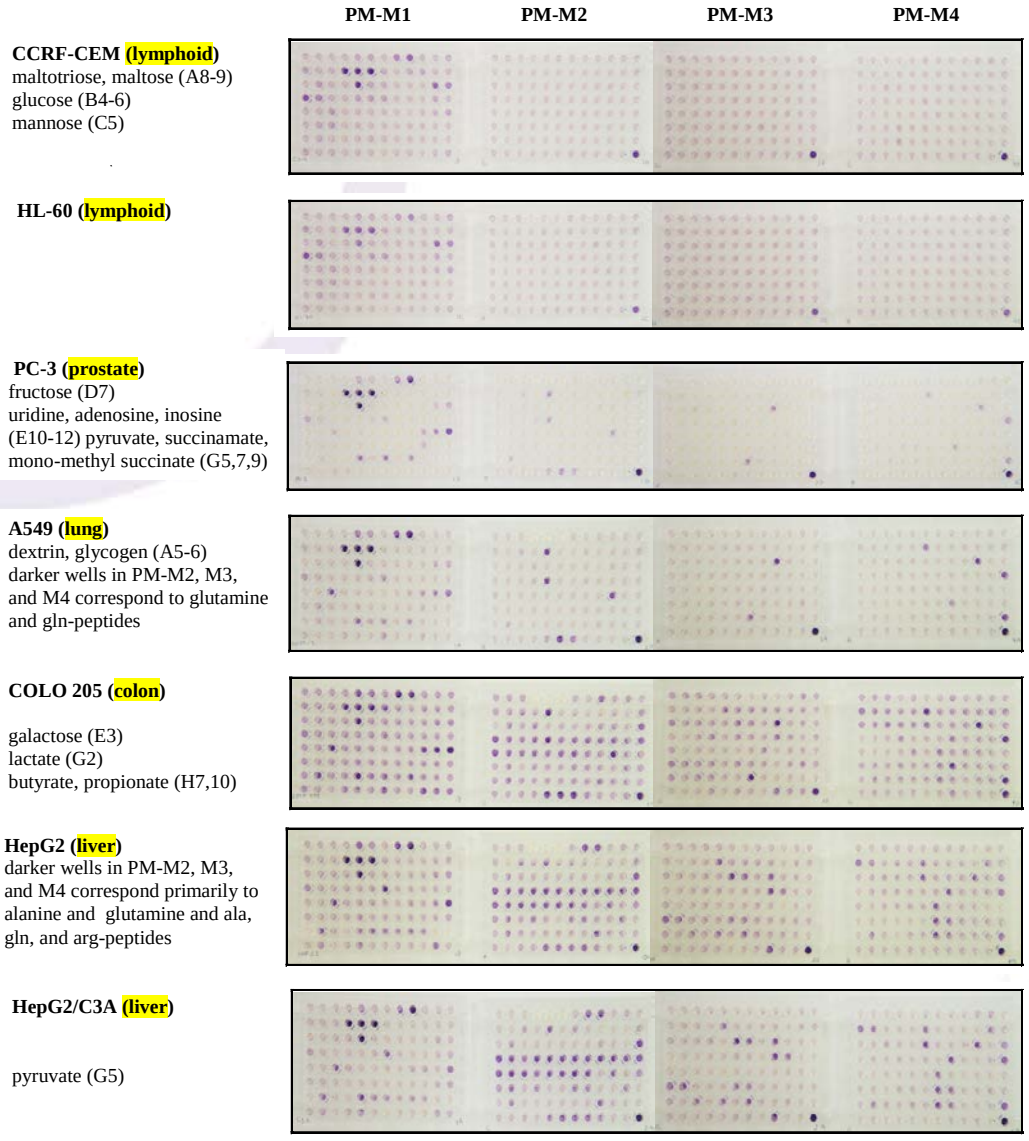
Cells Have Different Metabolic Phenotypes



Adapted from Bochner et. al. PLoS ONE (2011) 6:e18147

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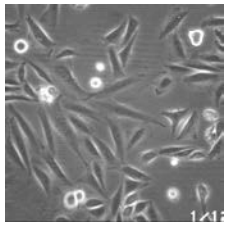
PMs Distinguish Closely Related Sublines

HepG2  vs HepG2/C3A, a subline created for an artificial liver project

PM-M1 PM-M2 PM-M3 PM-M4

HepG2

HepG2/C3A

pyruvate

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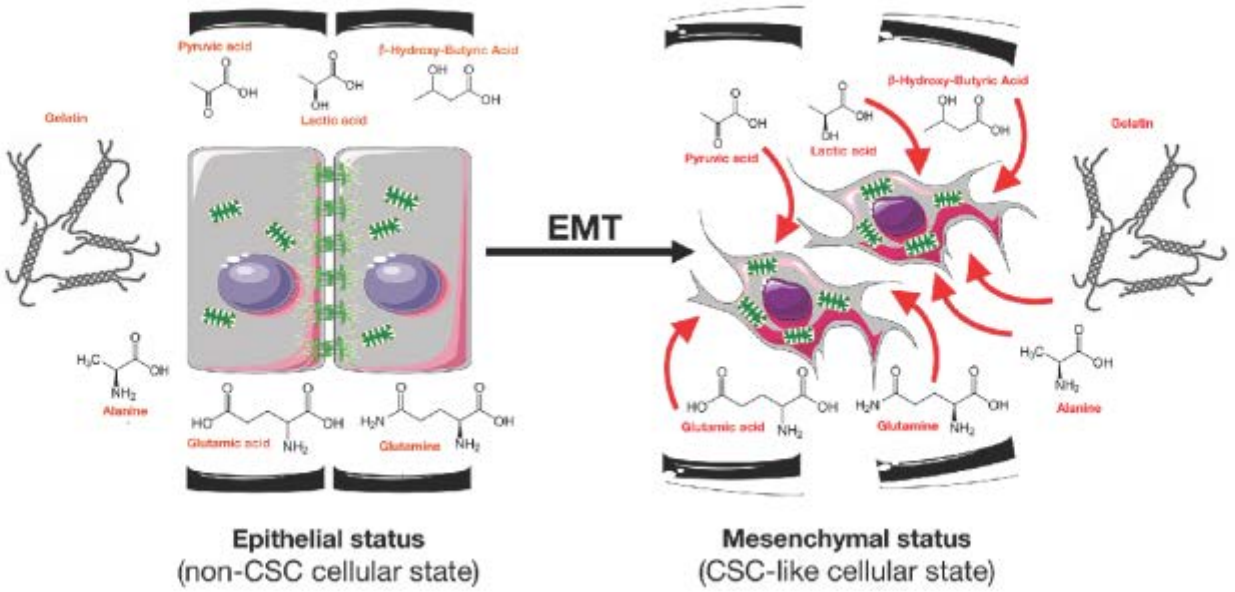
PM Comparison of Breast Cancer vs Cancer Stem Cell

Using the method of Robert Weinberg, cells can be induced to undergo an epithelial to mesenchymal transition (EMT) with stem cell-like properties



Isogenic stem cells were induced by inhibiting expression of the CDH1 E-cadherin gene via short hairpin RNA as described in

E Cuyas, B Corominas-Faja, JA Menendez, Oncotarget (2014) 5:3970

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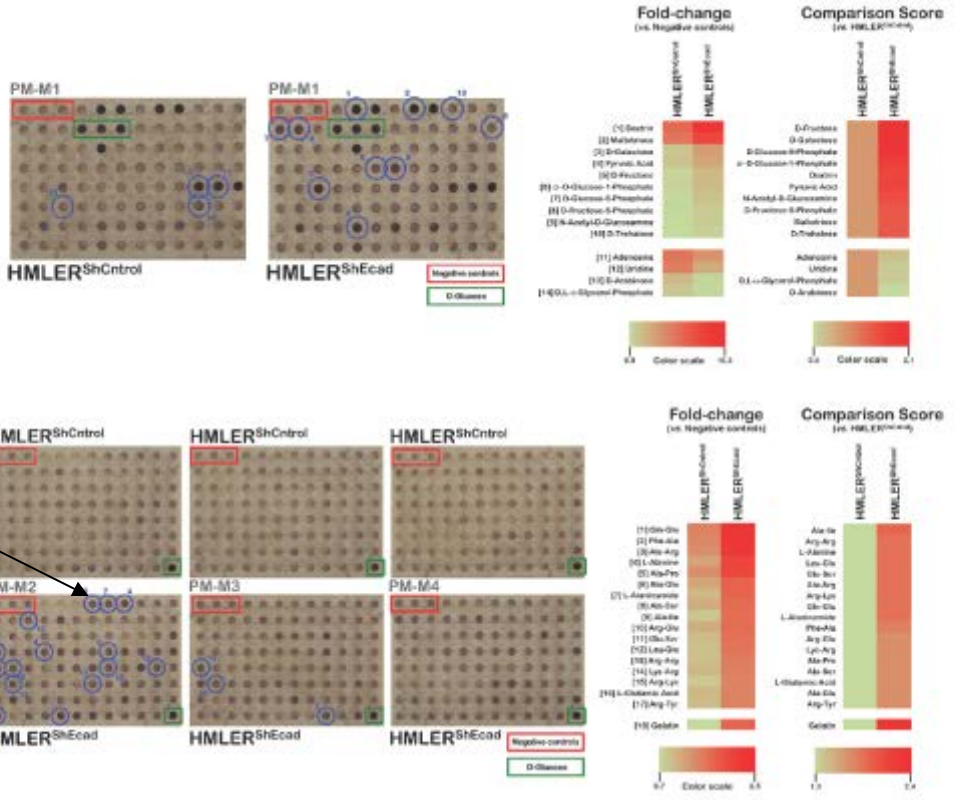
PM Comparison of Breast Cancer vs Cancer Stem Cell

"Most changes that occurred following the acquisition of a CS-like cellular state (28 out of 31, 90%) were increases in the ability to generate energy"



Induced metabolism of gelatin, amino acids, and peptides

Isogenic stem cells were induced by inhibiting expression of the CDH1 E-cadherin gene via short hairpin RNA as described in

E Cuyas, B Corominas-Faja, JA Menendez, Oncotarget (2014) 5:3970

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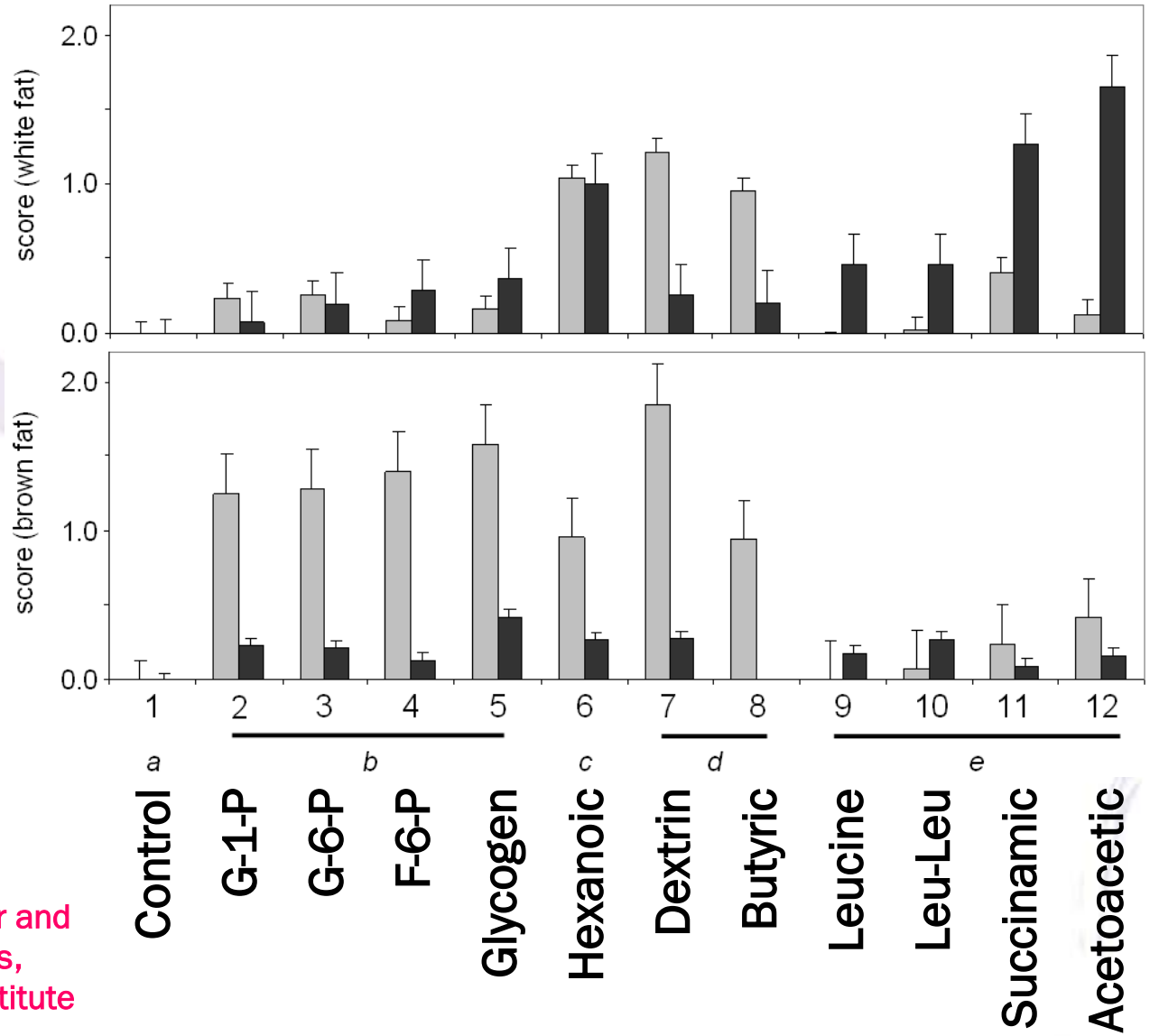
Metabolic Differences in Preadipocytes and Adipocytes



white fat

brown fat

Data of
B. Wagner and
P. Clemons,
Broad Institute

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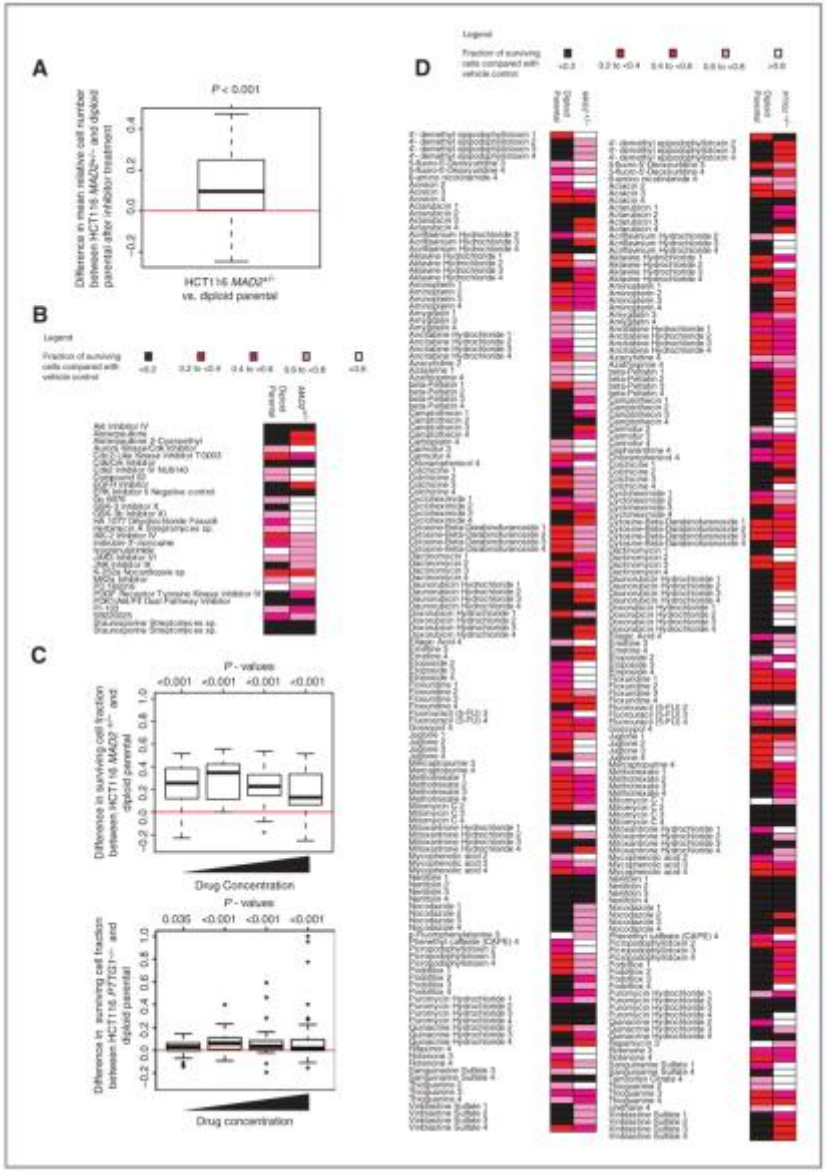
Resistance to Anti-Cancer Drugs in CIN+ Cancers



From Lee et. al. Cancer Res.(2011) 71:1858

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Examples of Cell Line Instability Detected by Metabolic Phenotype Analysis

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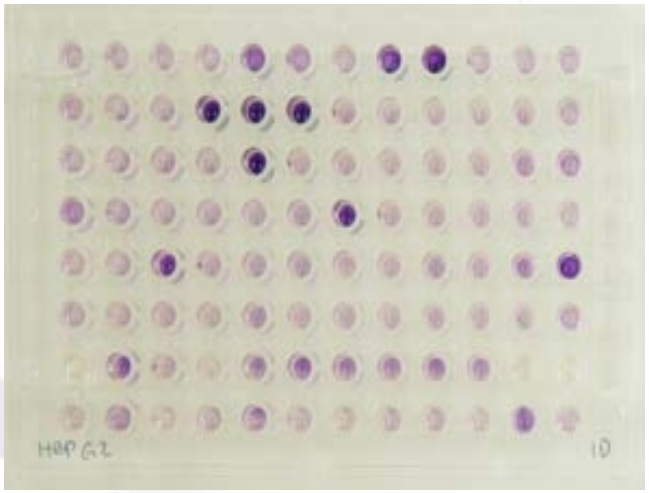
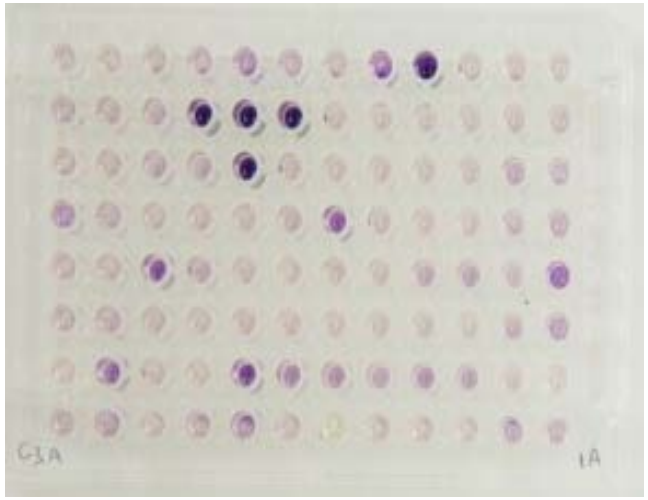
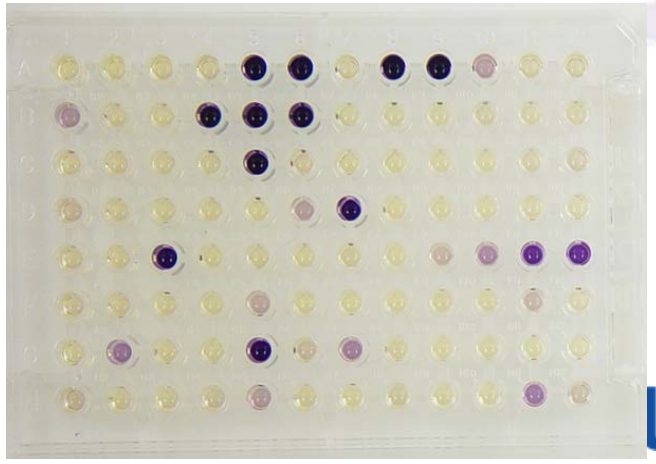
Modification of a Hepatocyte Cell Line

HepG2

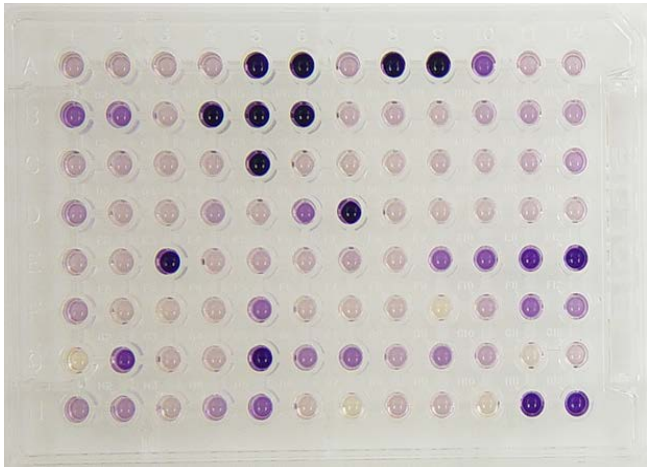




1

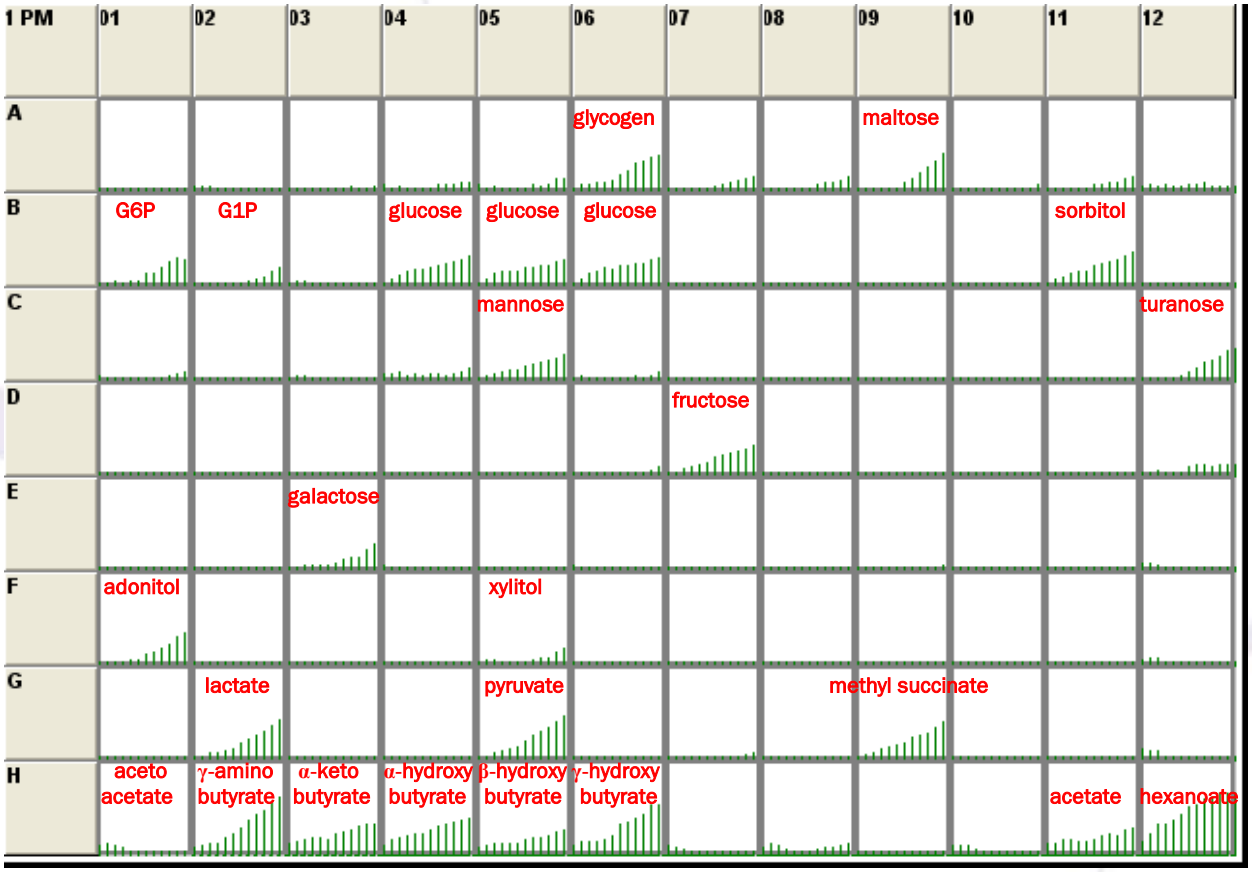


2



3

Detailed Metabolic Analysis of Primary Hepatocytes



Primary rat hepatocytes, 20,000 per well,
incubated for 3 hours

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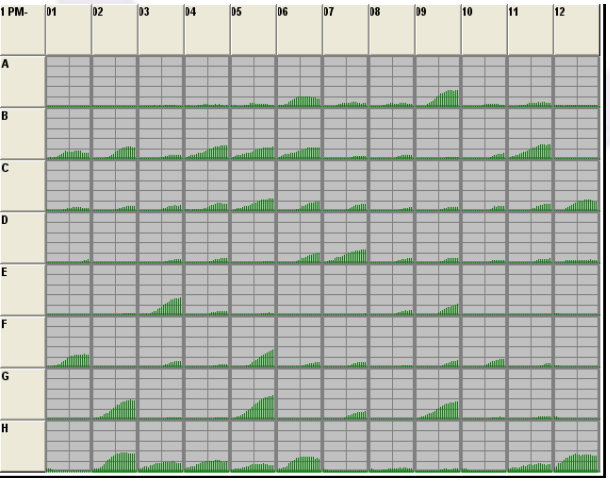
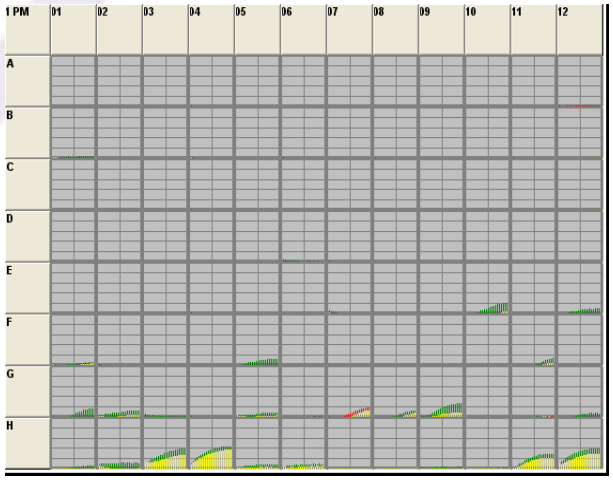
Hepatocyte Preps Show Lot-to-Lot Metabolic Differences

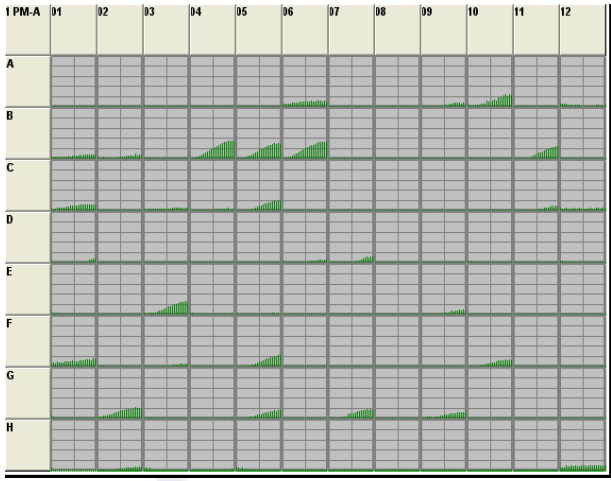
Lot 208



Lot 212



Lot 256



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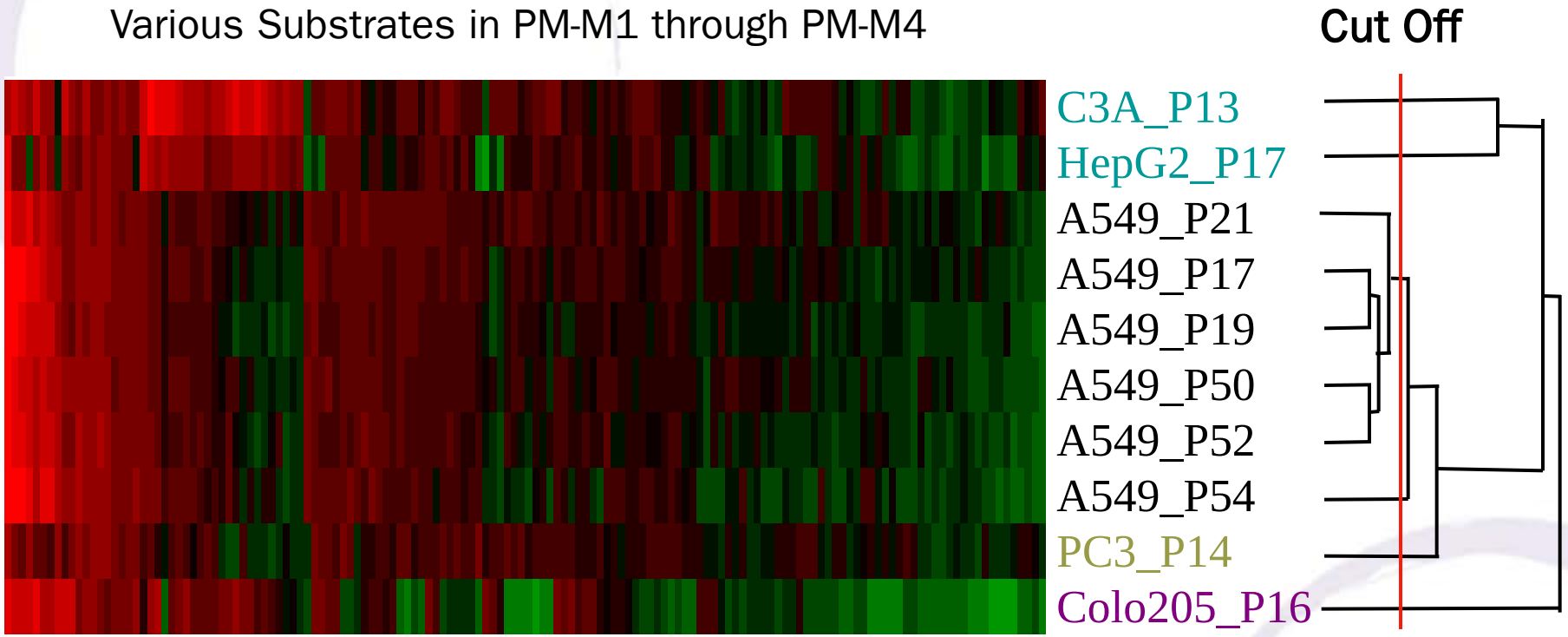
PMs Detect Changes with Cell Passaging

Various Substrates in PM-M1 through PM-M4



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

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Issues and Limitations of DNA-based QC

Wide ranging cell stability from unstable stem cells to relatively stable cell lines that have been maintained in artificial culture for decades.

DNA-based QC would not be a preferred method for characterizing and distinguishing

- cells of different tissue/organ type
- cells with point mutations
- ips reprogrammed cells, stem cells, differentiated cells
- cells with chromosome instability
- cells with epigenetic changes
- cells with metabolic changes

<u>All of these can potentially be detected by analysis of metabolic and other phenotypes</u>

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Other Reasons for Performing Metabolic QC

Cell Metabolism based Bioprocess Production

- CHO cells producing recombinant proteins
- Hybridoma cells producing monoclonal antibodies
- Yeast producing special wines
- Bacteria producing antibiotics
- Bacterial cocktails producing special yogurts or probiotics for faecal transplants.

Cell-based Assays

- Primary liver cells used in tox assays

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Summary

- There is no perfect solution or right answer to how much and what type of cell line QC should be performed. It is a judgement call based on assessment of how stable the cell is and how best to detect changes that it is prone to make.
- In general, it is prudent to perform a metabolic/phenotypic QC in addition to a genetic QC as these are complementary analyses.
- Biolog phenotypic assays provide an ideal metabolic/phenotypic platform that spans the spectrum of cells from bacteria to human cells.
- Biolog assays are very easy and inexpensive to perform and they can be read with any microplate reader or even by eye. However the best data is obtained by measuring metabolic rates using the OmniLog instrument.

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The Importance of Authentication

Methicillin-resistant *Staphylococcus aureus*

-Photo courtesy of NIAID

- The use of minimally cultured strains that have been propagated and preserved under the proper conditions can help prevent:
  - Genetic drift
  - Phenotypic variation
  - Changes in functional characteristics
  - Contamination
  - Misidentification

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- **November 12, 2015**
  10:00 AM, 3:00 PM EST
  Bill Hirt, Ph.D., *Director of Accreditation*, ANAB
  How Does ISO 17025 Accreditation Build International Confidence?

Please email additional questions to:

tech@atcc.org

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