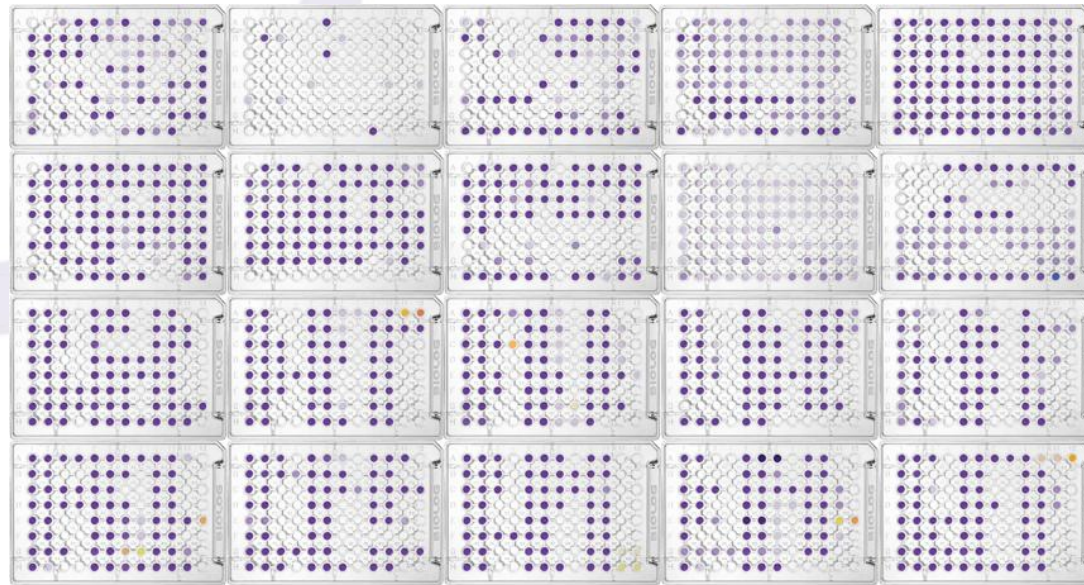


Phenotype MicroArrays: A Platform for Phenotypic Characterization of Cells and Species Description



Stacy O. Montgomery, Ph.D.
ICCC12 – Florianopolis Brazil
30 Sept. 2010
smontgomery@biolog.com

Agenda

Microbial Identification (MI)

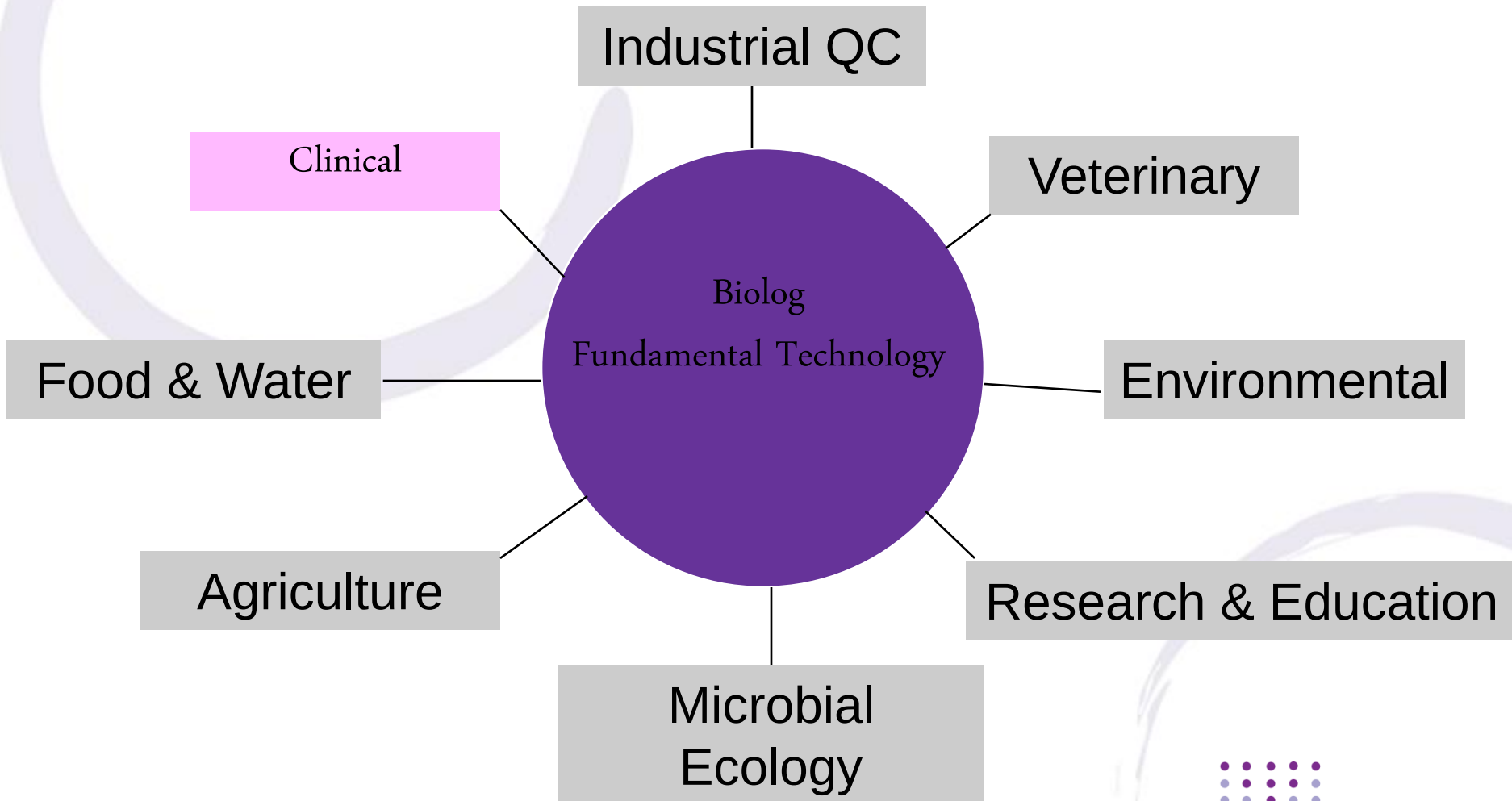
- Fundamental Technology

Phenotype MicroArray (PM) Technology

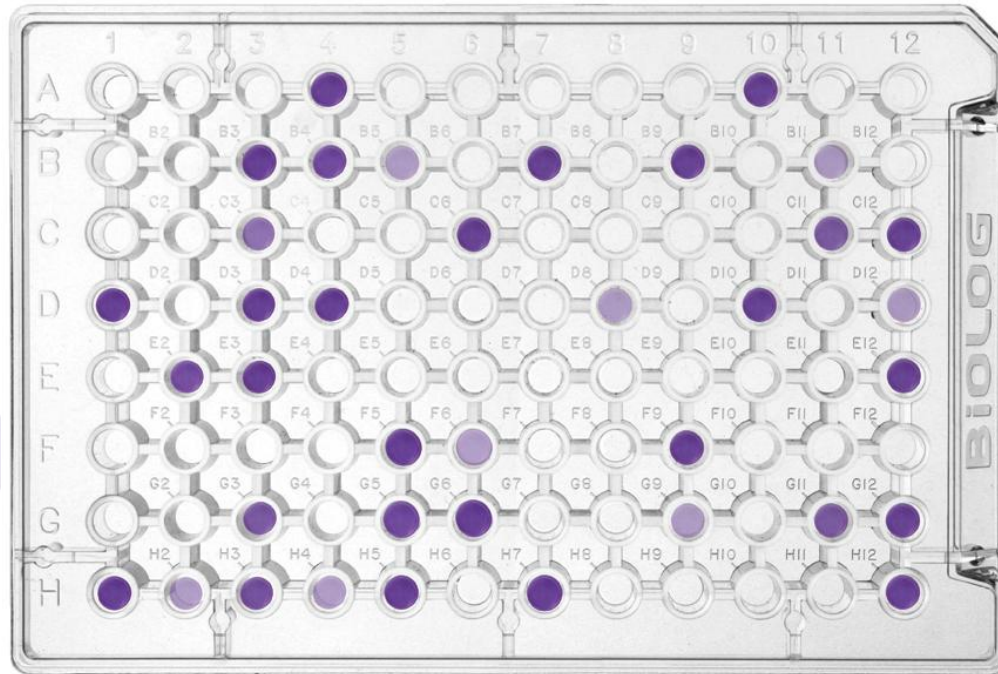
- PM Technology for Gene Function
- PM Technology for Drug Discovery
- PM Technology for Strain Characterization

Fungal Applications

Primary Markets



Chemistry Platform: Carbon Utilization



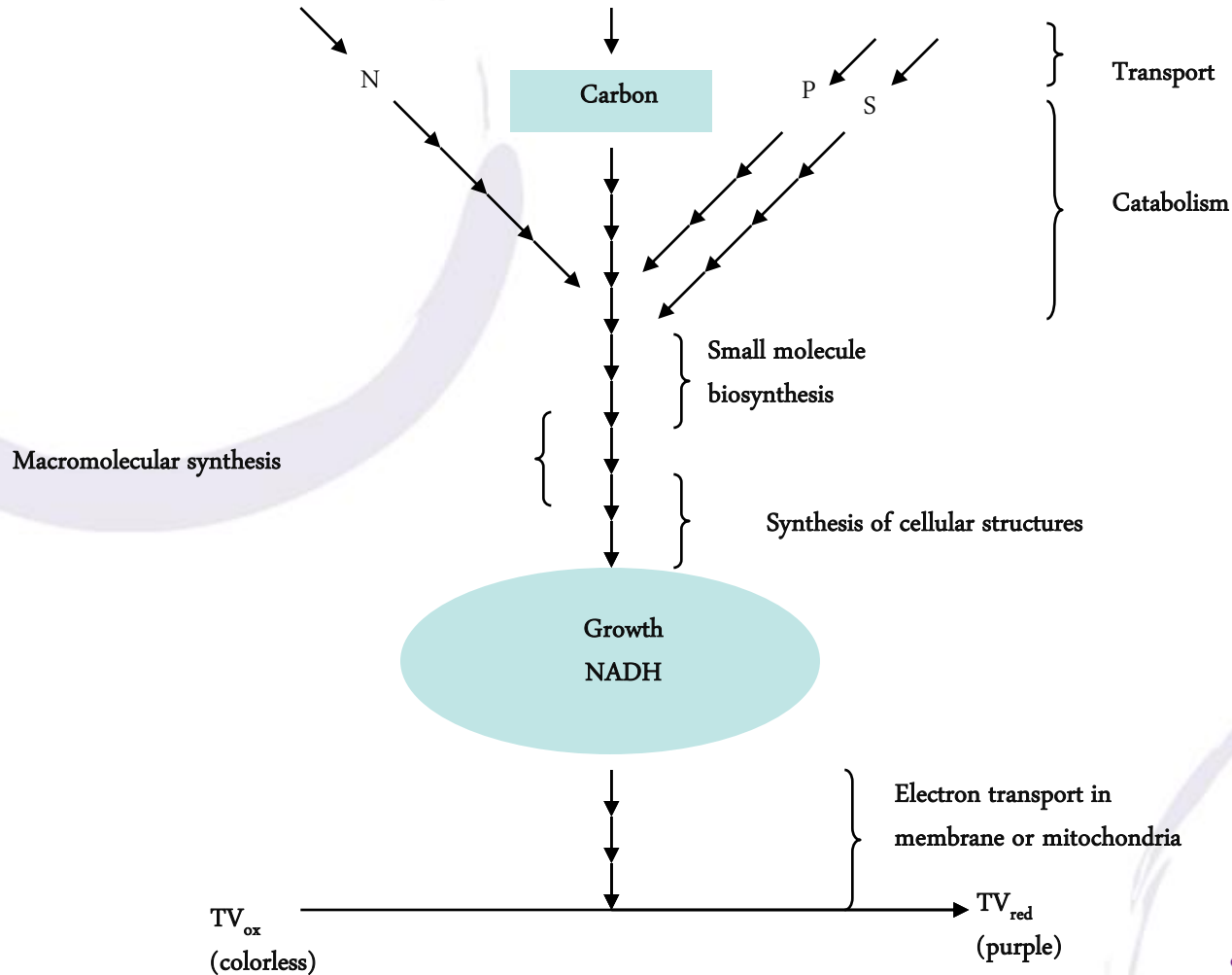
Patterns begin developing in as little as 4 hours

Species are DEFINED by Patterns of Utilization

TABLE BXII.β.2. Utilization of **carbon** compounds by some *Burkholderia* species^{a,b}

Compound ^c	<i>B. cepacia</i>	<i>B. andropogonis</i>	<i>B. caryophylli</i>	<i>B. coovenensis</i>	<i>B. gladioli</i>	<i>B. glathei</i>	<i>B. glumae</i>	<i>B. graminis</i>	<i>B. mallei</i>	<i>B. phenazinium</i>	<i>B. plantarii</i>	<i>B. pseudomallei</i>	<i>B. pyrocinia</i>	<i>B. vandii</i>	<i>B. vietnamiensis</i>
<i>Carbohydrates/glycosides:</i>															
N-Acetylglucosamine	+	-	+		+	+	+	+	+	+	+	+	+		+
Amygdalin	+	-	-		-	-	d ⁻	-		-	+		+		d ⁺
D-Arabinose	+	+	+		+	+	+	+	+	+	+	+	+		+
L-Arabinose	+	+	+	+	+	+	+	+	d	+	+	-	+		+
Arbutin	+	-	-		-	-	d ⁻	-		-	+		-		d ⁺
Cellobiose	d ⁺	-	d ⁻	+	d ⁺	-	d ⁺	d	+	-	+	+	+		+
L-Fucose	+	-	+	+	+	+	+	+	+	+	+	+	+		+
D-Fucose	+	d ⁻	+	+	+	+	d ⁺	d	+	-	+	+	-		+
Gentiobiose	d ⁺	-	d ⁻		-	-	d ⁻	d		-	+		+		+
Glucosamine	+	-	+		+		+				+				+
2-Ketogluconate	+	d ⁻	+		+	+	+	+	d	+	+	+	+		+
5-Ketogluconate	+	-	+		+	+	d	+		+	+		+		-
Lactose	-	+	-		-	+	-	+	-	-	-	-	-	+	d ⁻
D-Lyxose	+	+	+	-	+	+	+	+		-	+		+		+
Maltose	d ⁻	-	-	-	-	-	-	-	d	-	-	+	-		-
Melibiose	d ⁻	-	-	+	-	-	+	-		-	-		-		-
Raffinose	d ⁺	-	+	-	-	-	d ⁺	+		-	-		-		d ⁺
L-Rhamnose	d	d ⁺	+	-	-	+	-	+	-	+	±	-	-	-	-
D-Ribose	+	+	+	+	+	-	+	d	-	+	+	+	+		d ⁻
Salicin	+	-	-	+	-	-	d ⁻	-	d	-	+	+	-		d ⁺
Sucrose	d ⁺	-	+	-	-	+	-	+	+	+	±	+	+	-	+
Tagatose	+	-	-		+	+	d ⁺	-		-	-		+		+
Trehalose	+	d ⁻	+	+	+	-	+	+	+	-	-	+	+	+	+
D-Xylose	d ⁺	-	+	+	+	+	+	+	+	+	+	-	+	+	+

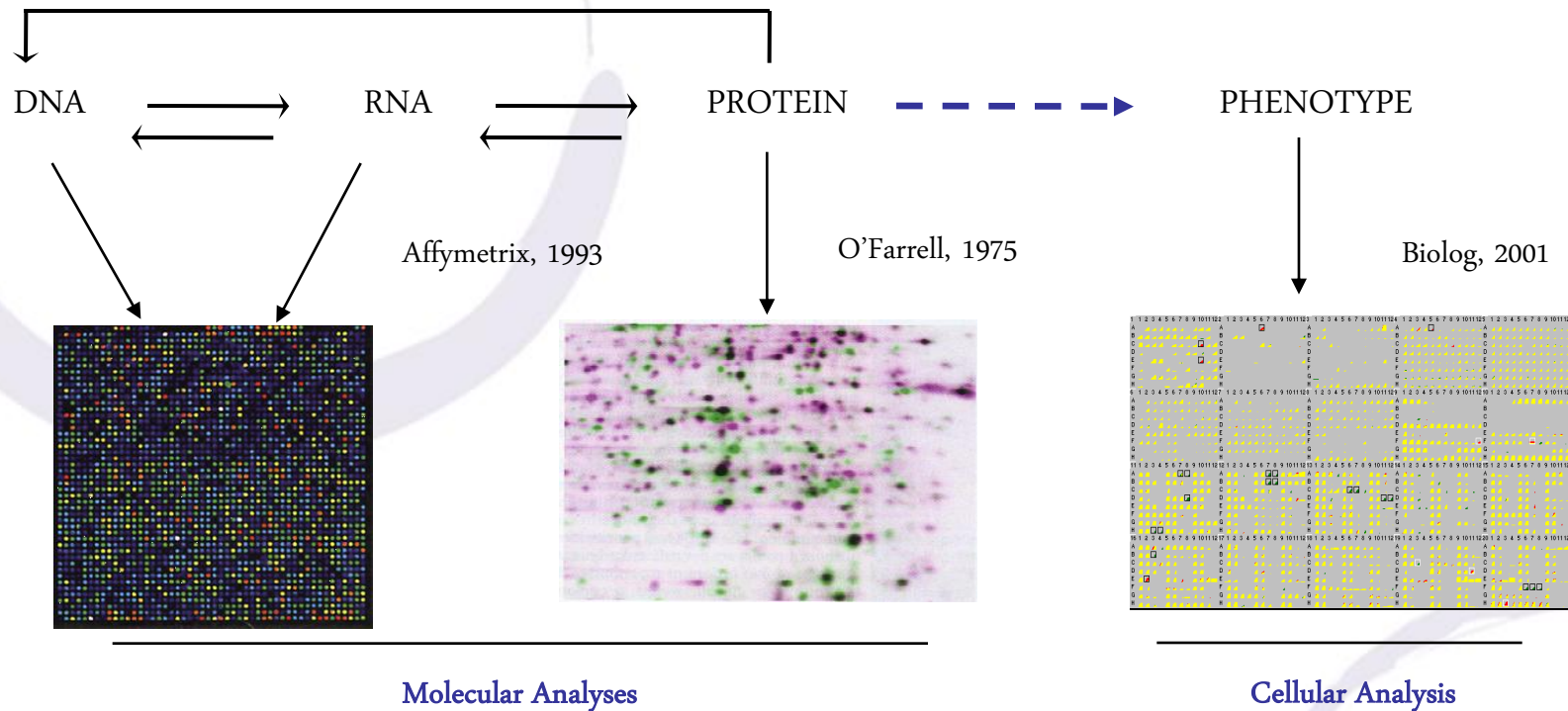
Universal Reporter System - Respiration



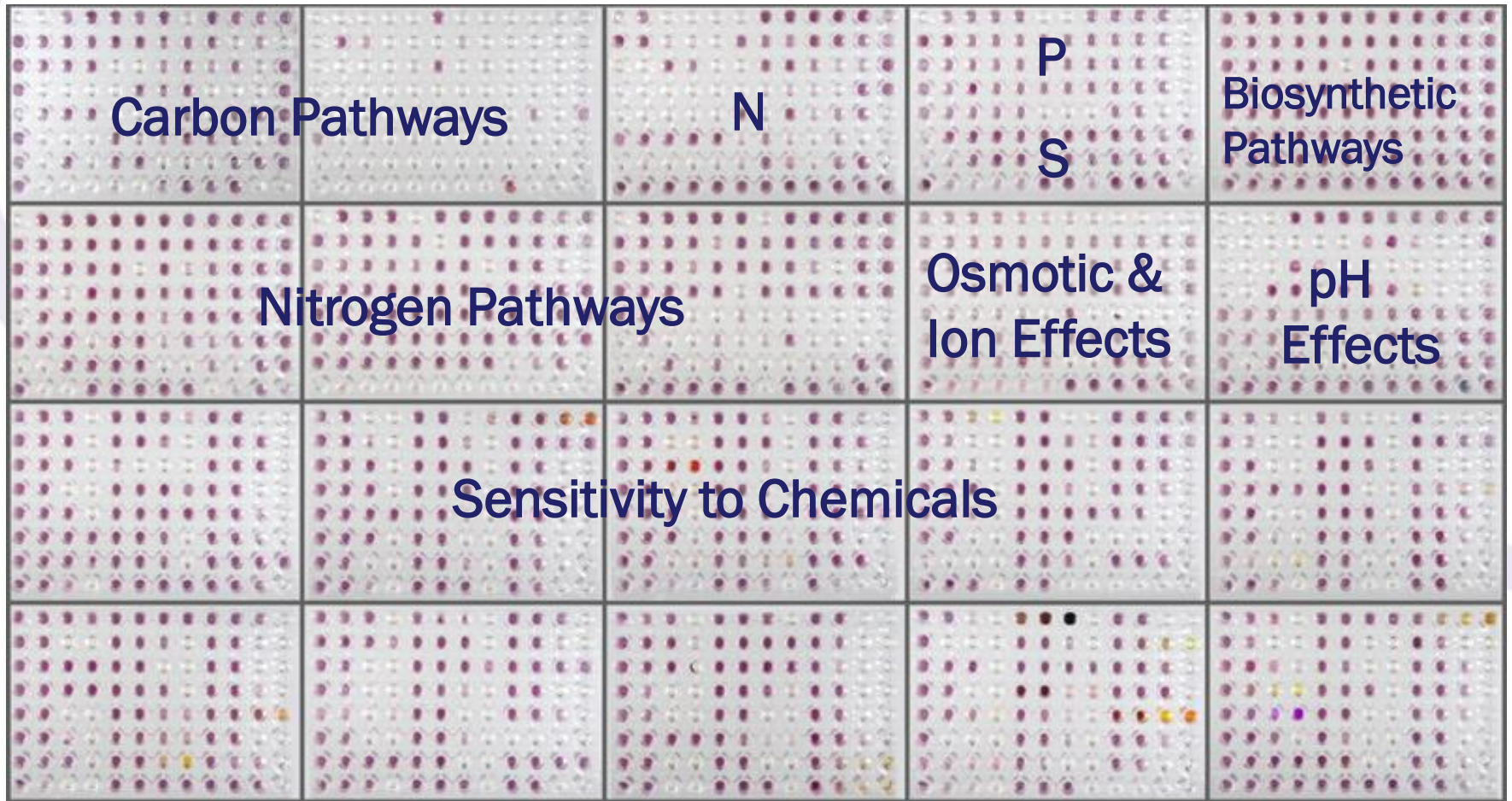
Biolog GEN III System

- No pre-categorization: no gram stain, oxidase, catalase
- No additional follow-on tests
- One test panel for both GN and GP
- One minute set up
- One color change
- Over 1300 taxa
- Provides biologically relevant information

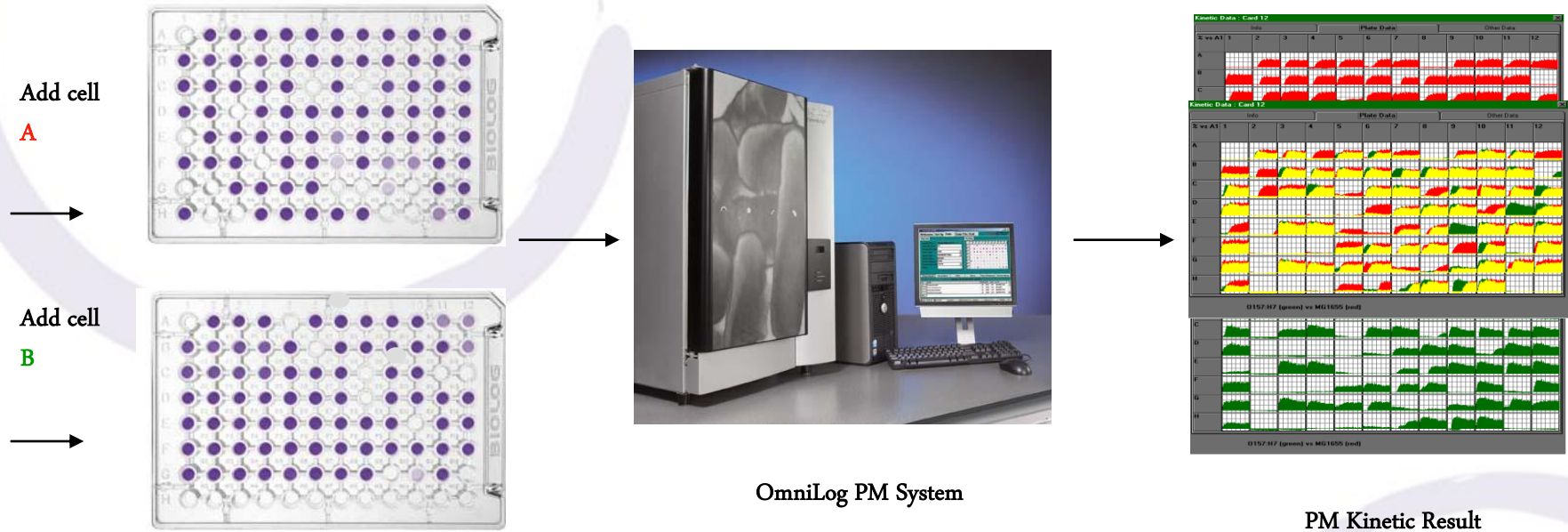
Why Measure Cellular Phenotypes?



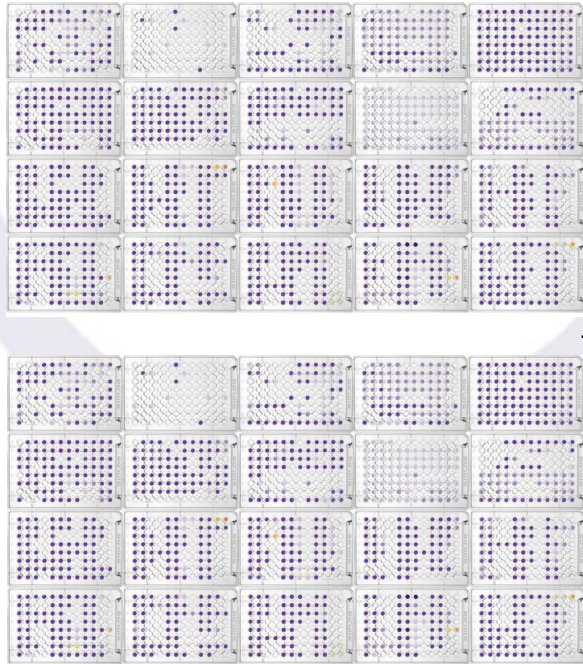
Measure 2000 Phenotypes



Comparing Two Cell Lines



PM Platform – Comparing Two Cell Lines



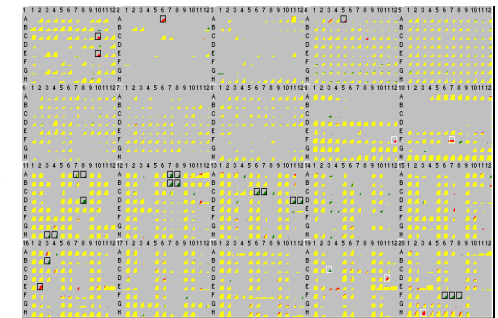
PM Pattern

1 hr



OmniLog PM System

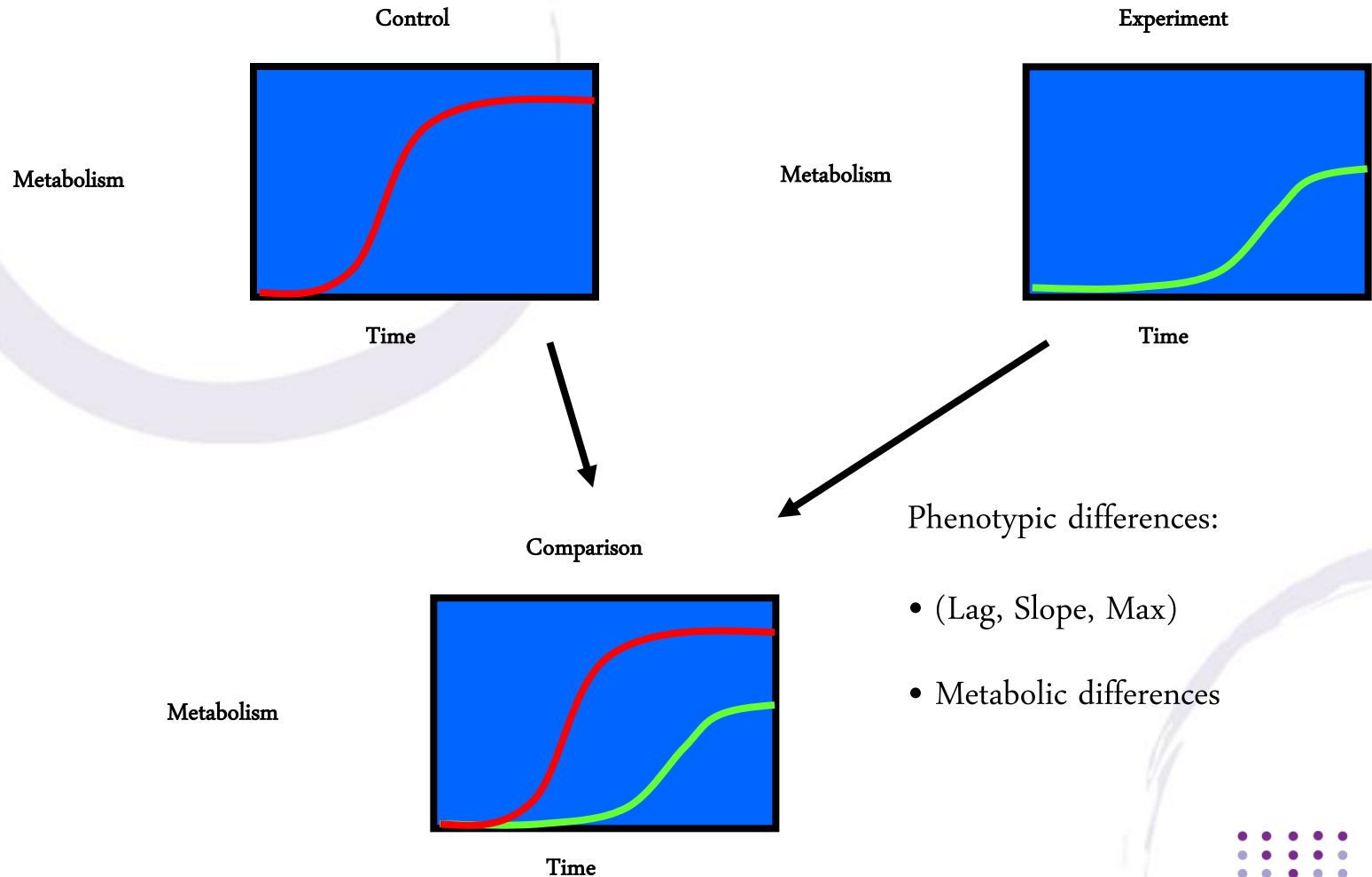
Automatic

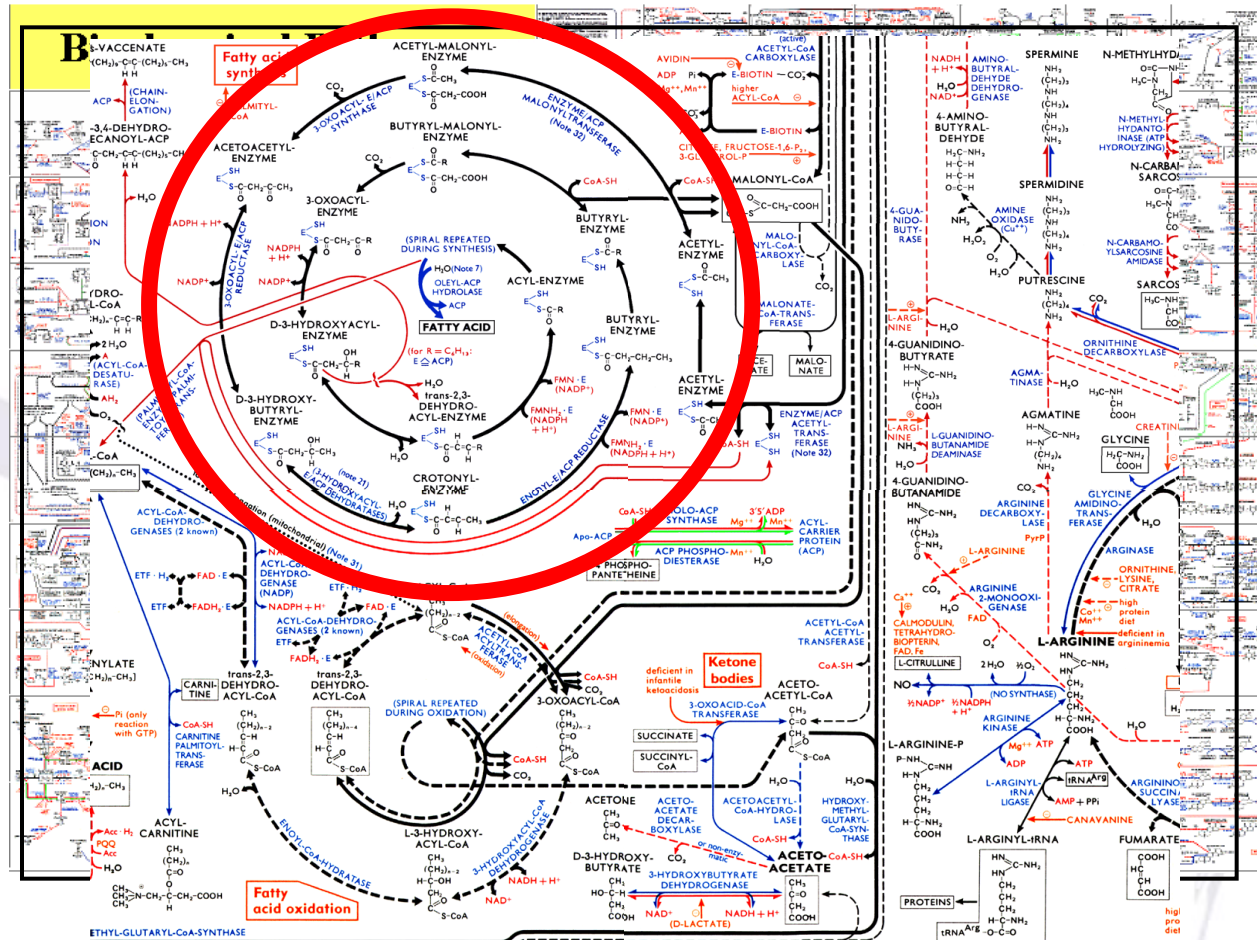


PM Kinetic Result

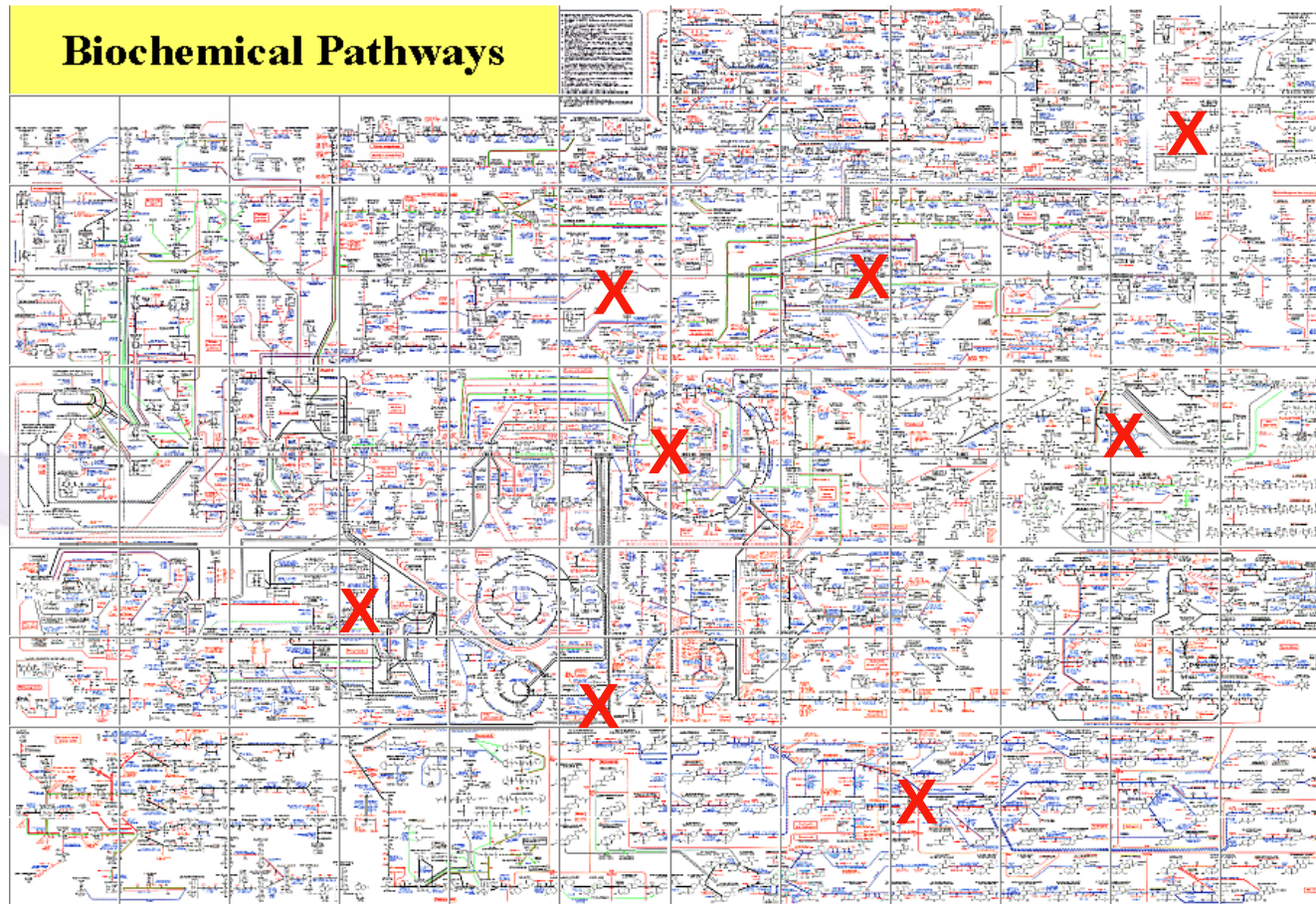
24-48 hr

Metabolic Curves Compared





Inhibitors Knockout Various Pathways



Phenotype MicroArray™ Applications

- Testing Cell Lines with Genetic Differences
 - Determining Gene Function
- Testing Cell Lines Exposed to Drugs / Chemicals
 - Evaluating New Drug Candidates
- Direct Testing of Cell Lines
 - Strain Description
 - Strain Characterization
 - Optimizing Growth Conditions / Production Characteristics
 - Testing Cells for Phenotypic Stability
 - QA / QC of cell lines

Phenotype MicroArray™

Testing Genetic Effects on Cells



Assaying Genetic Changes

Genotype → **Phenotype**

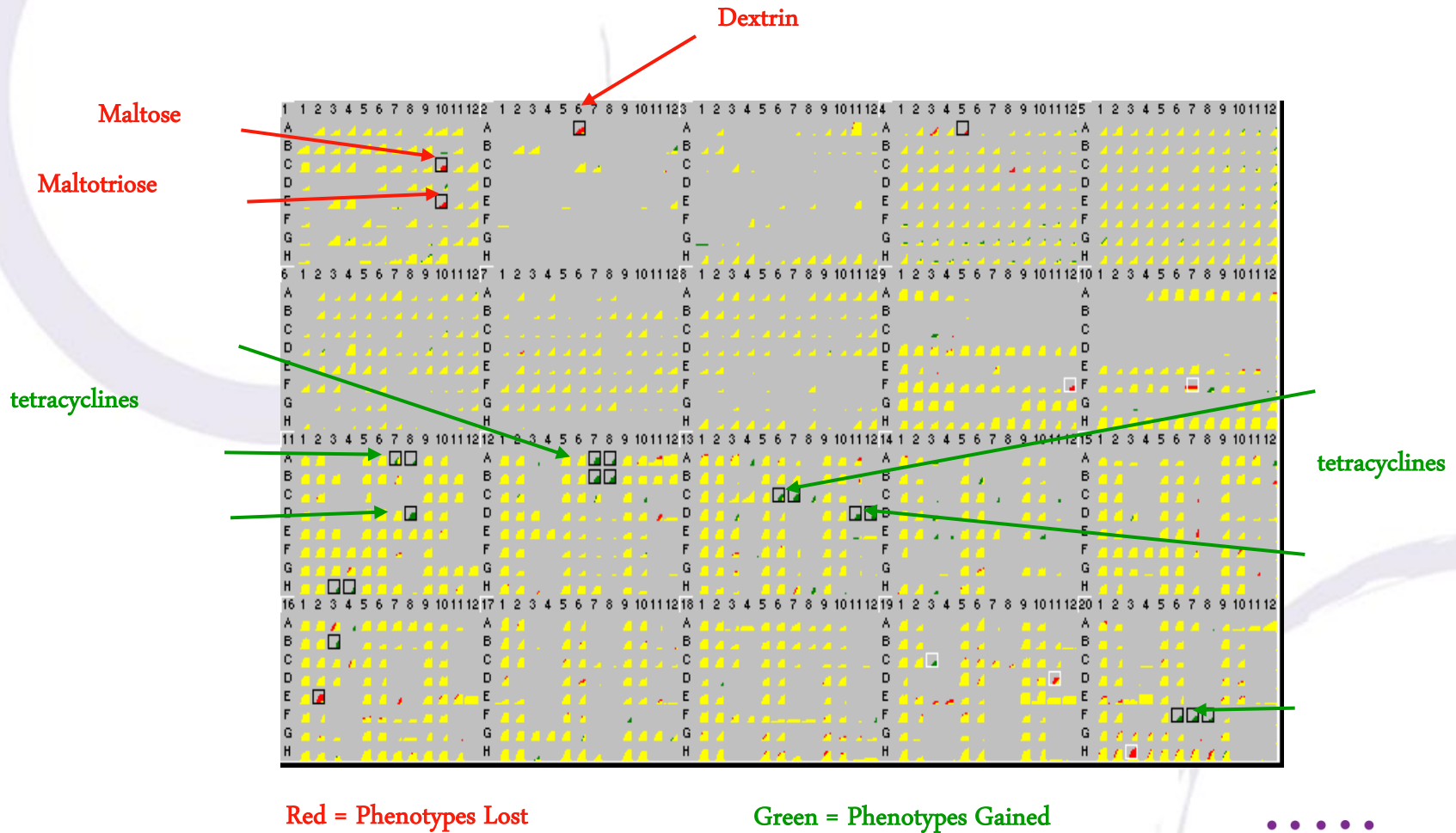
Phenotype
MicroArrays

Knock out a gene

Which phenotypes change?

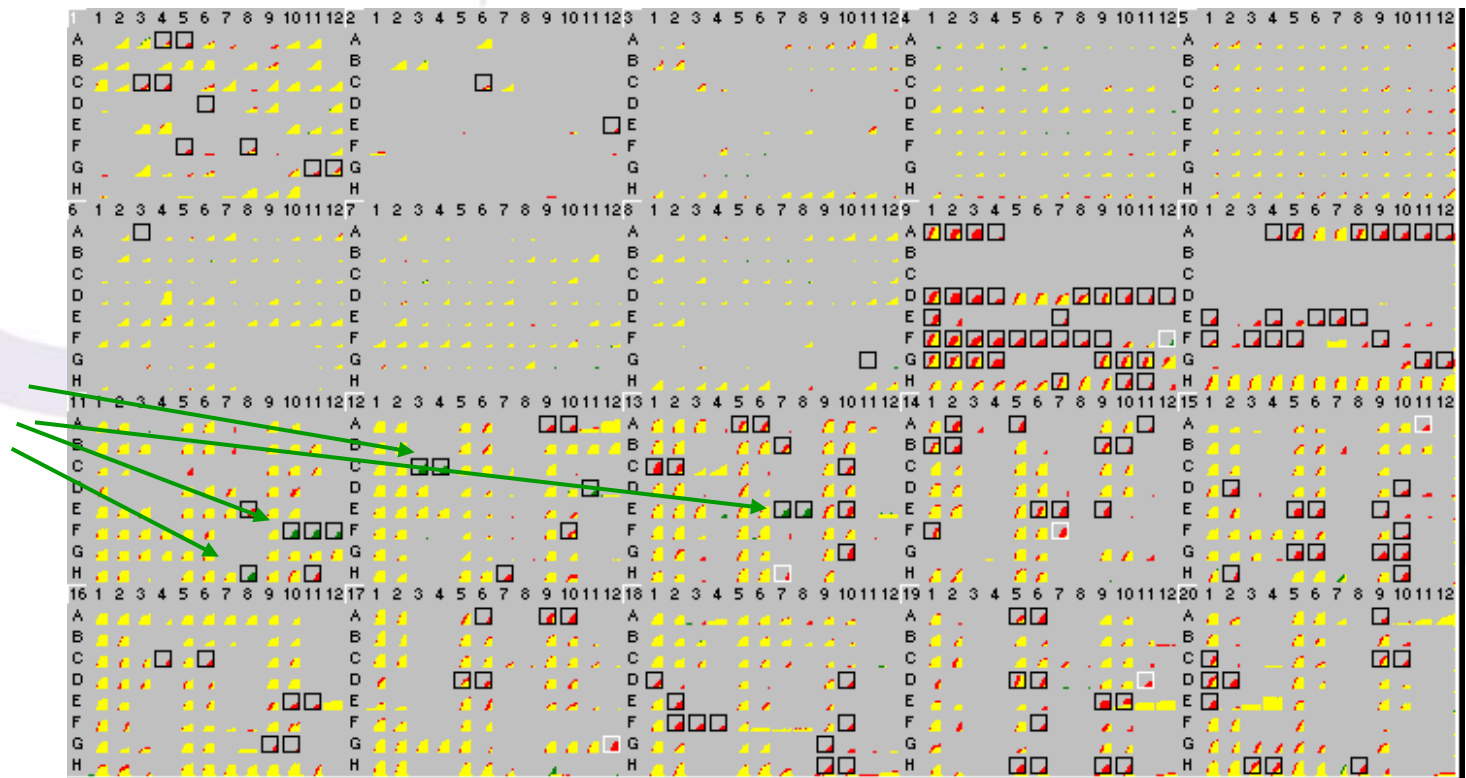
Compare Mutant to Wild Type to Determine Gene Function

E. coli malF::Tn10 vs MG1655



E. coli oxyR::kan vs MG1655

amino-
glycosides



Phenotype MicroArrays™

Direct Testing of Cell Lines

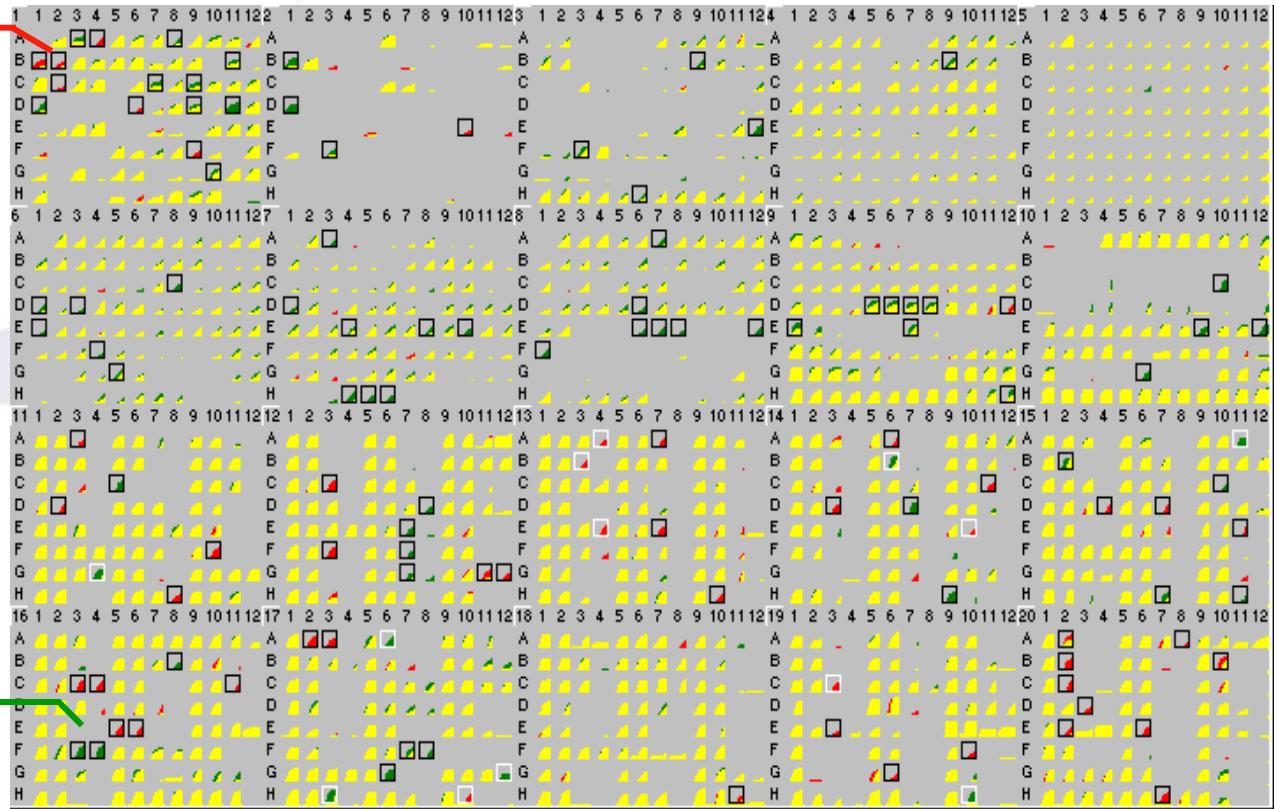


Comparison of Strains

Pathogenic (0157) and non-Pathogenic (MG1655) *E. coli*

sorbitol

tellurite



Culturing an “Obligate Intracellular Pathogen”

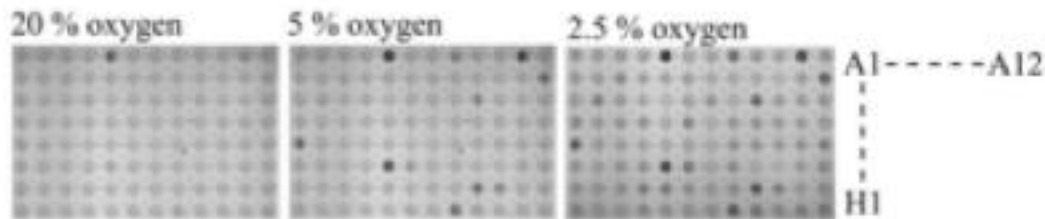
Host cell-free growth of the Q fever bacterium *Coxiella burnetii*

Anders Omsland^a, Diane C. Cockrell^a, Dale Howe^a, Elizabeth R. Fischer^b, Kimmo Virtaneva^c, Daniel E. Sturdevant^c, Stephen F. Porcella^c, and Robert A. Heinzen^{a,1}

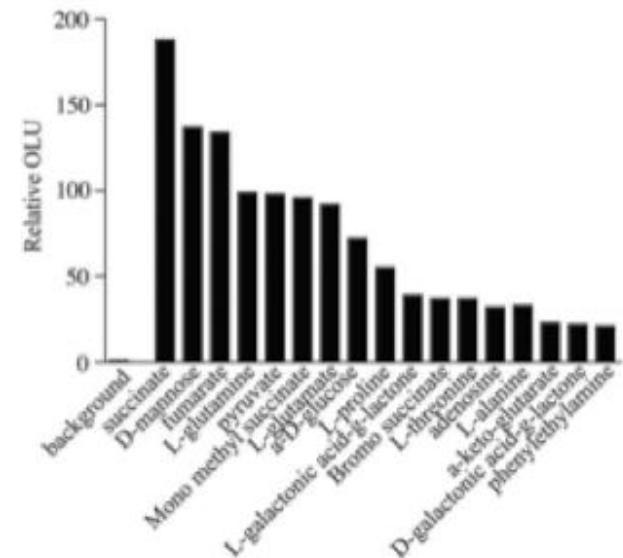
^aCoxiella Pathogenesis Section, Laboratory of Intracellular Parasites, ^bElectron Microscopy Unit, and ^cGenomics Unit, Research Technology Section, Research Technology Branch, Rocky Mountain Laboratories, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Hamilton, MT 59840

Edited by Emil C. Gotschlich, The Rockefeller University, New York, NY, and approved January 22, 2009 (received for review November 26, 2008)

B



C



Annotation of Transporter Genes in *P. aeruginosa*

- Ian Paulsen and coworkers (PLOS Genetics, Sept. 2008) examined phenotypes of knockouts of transporter genes and compared them with functional annotations based on DNA homology.
- Only 12/27 (44%) precisely matched predicted annotation
- In 10/27 (37%) a more precise annotation was obtained
- In 5/27 (18%) a significant reannotation was enabled
- Novel transporters were identified for L-glutamate, N-acetyl-L-glutamate, hydroxy-L-proline, and histamine

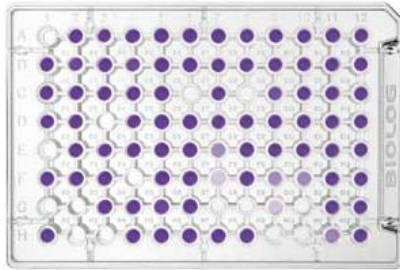
Phenotype MicroArrays™

Testing the Effects of Chemicals

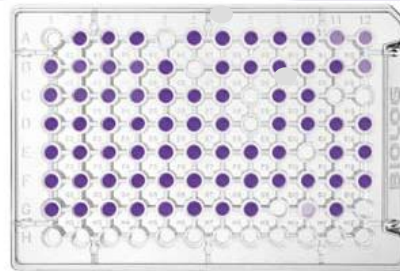


Testing & Evaluating Drugs

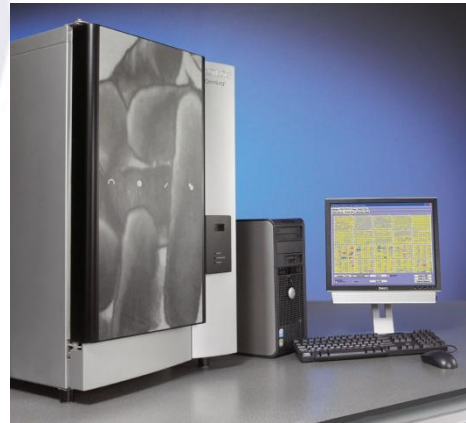
Add
cells



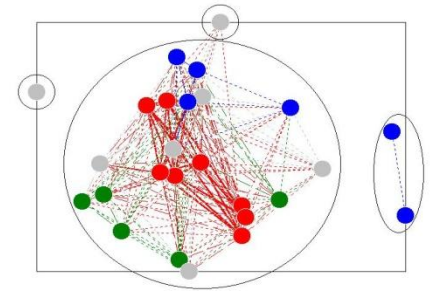
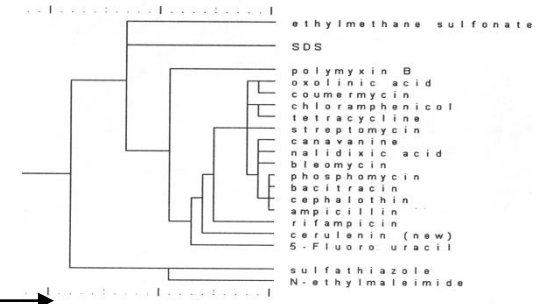
Add cells
+ drug



PM Pattern



OmniLog PM System



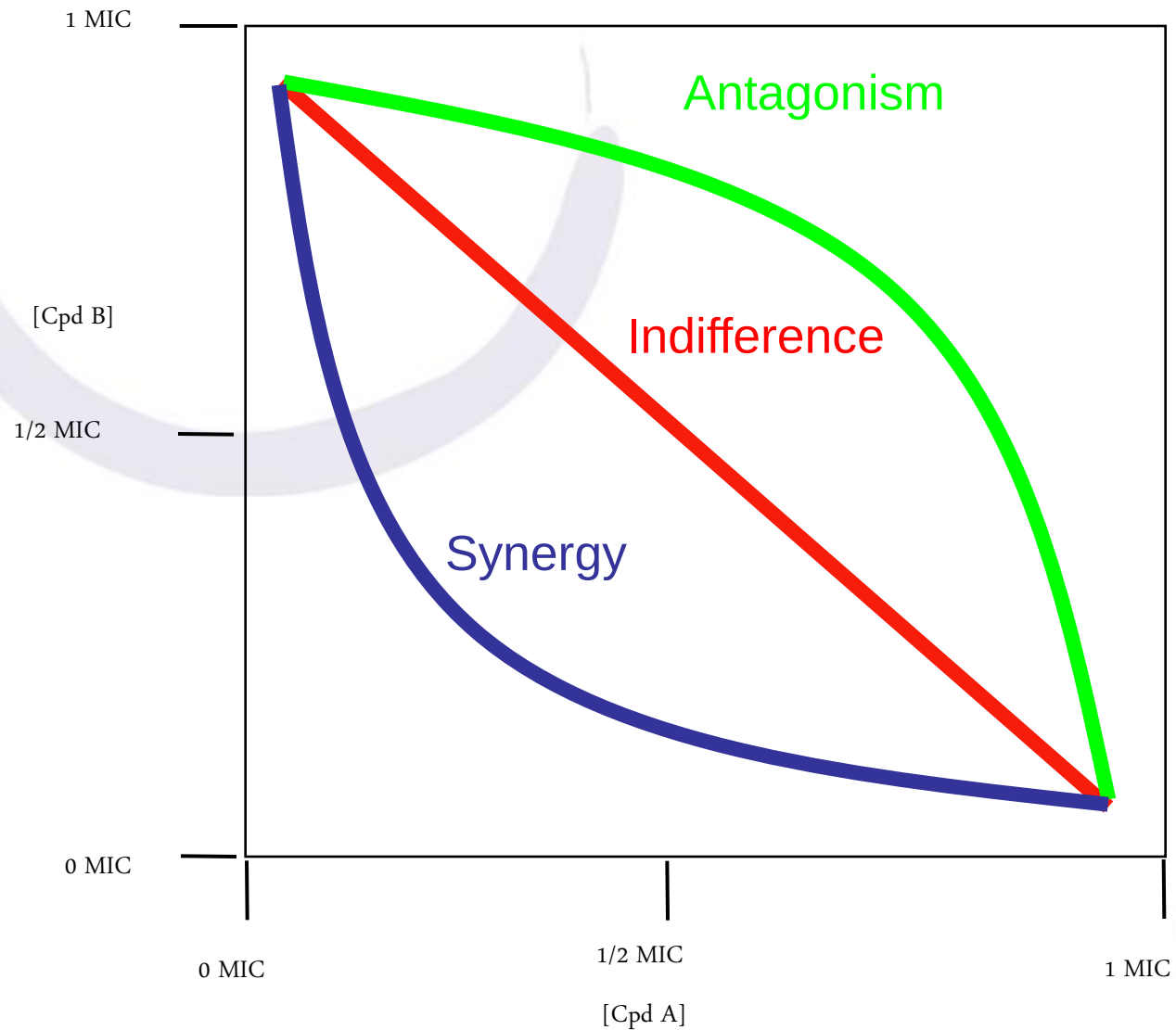
● Cell Wall
● DNA Gyrase
● Ribosome

——— < 1.3
- - - < 2.5
... < 3.8
..... < 5.0

Bioinformatics Analysis

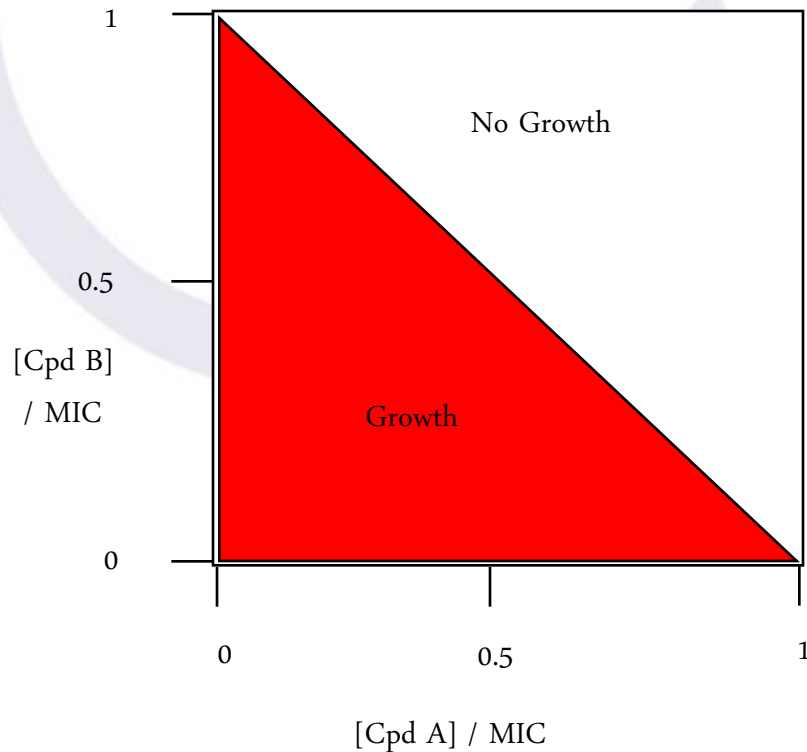
Patterns based on effect of drug under 2000 different growth conditions

Drug Interactions

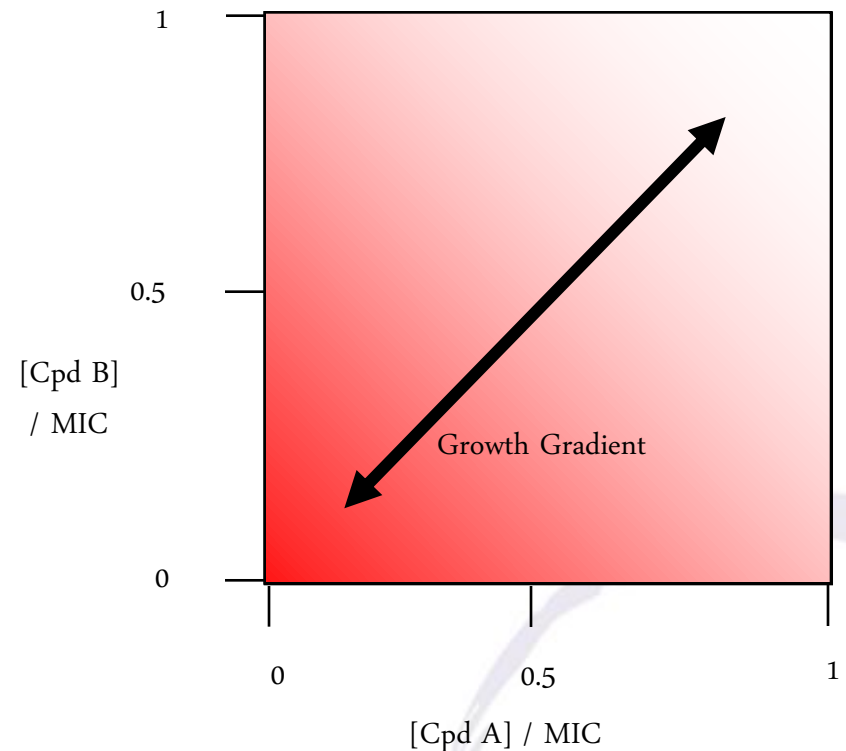


Isoblograms: Indifference

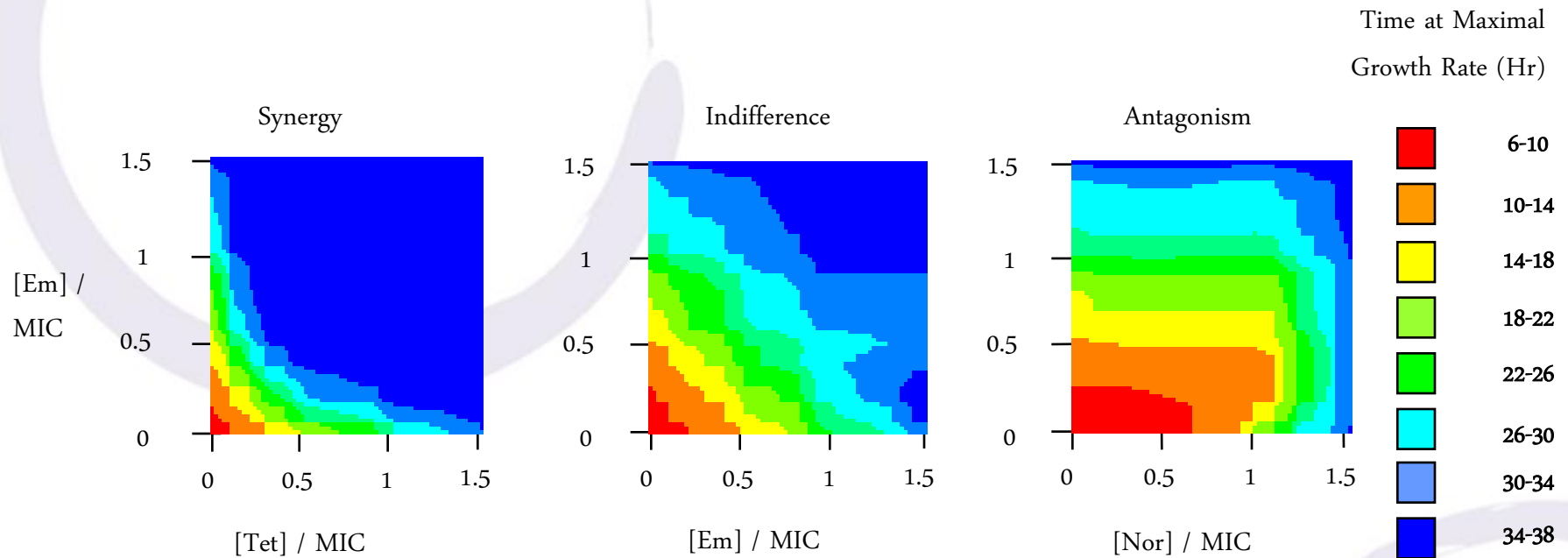
Classical



Gradient



Isobolograms of Antibiotics Tested in *S. aureus*

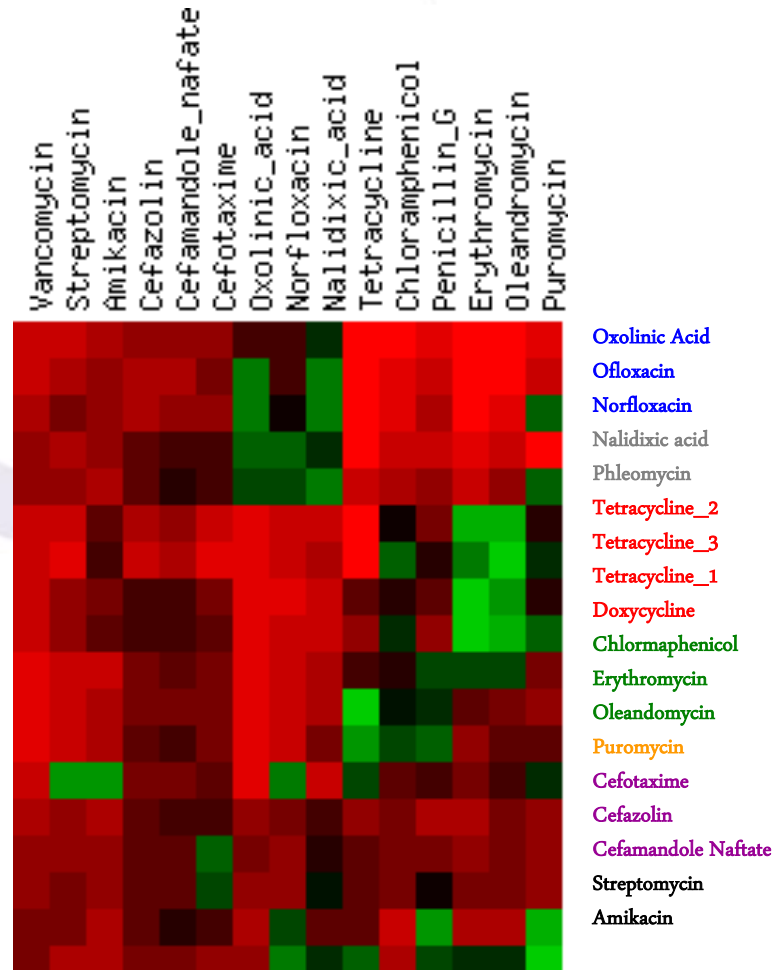


Em = Erythromycin

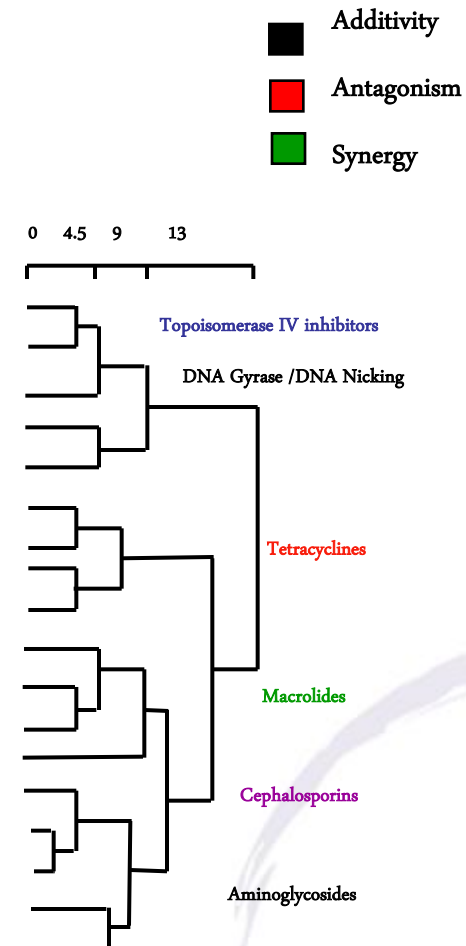
Tet = Tetracycline

Nor = Norfloxacin

Cluster Inhibitors into Groups



Similar Mechanisms of Action





Fungal Applications

Metabolic Profiling of Closely Related Fungi

Journal of Microbiological Methods 77 (2009) 102–108



Contents lists available at [ScienceDirect](#)

Journal of Microbiological Methods

journal homepage: www.elsevier.com/locate/jmicmeth



Application of Biolog FF MicroPlate for substrate utilization and metabolite profiling of closely related fungi

Maya Prakash Singh

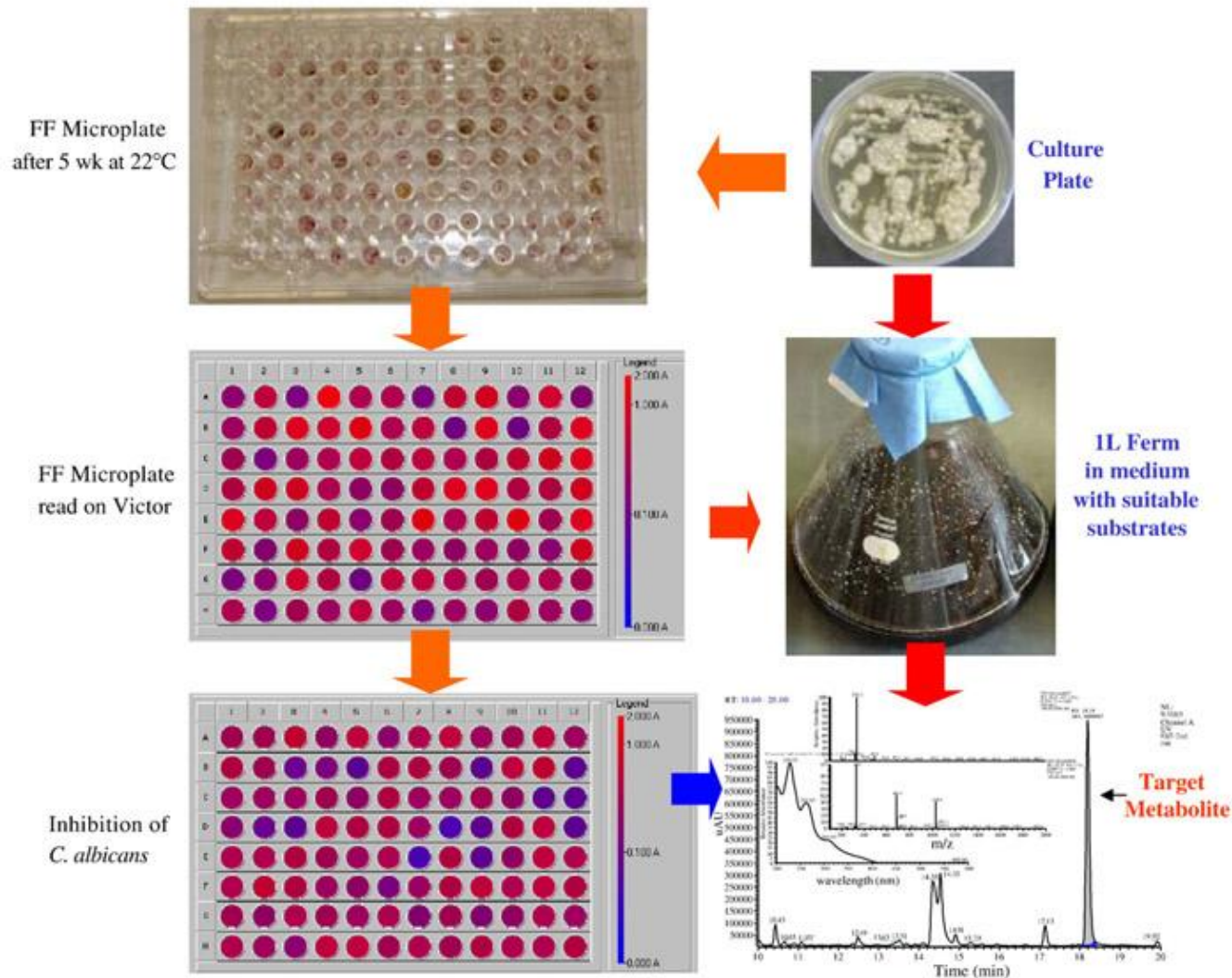
Chemical and Screening Sciences, Wyeth Research, Pearl River, NY 10965, United States



Metabolic Profiling of Closely Related Fungi

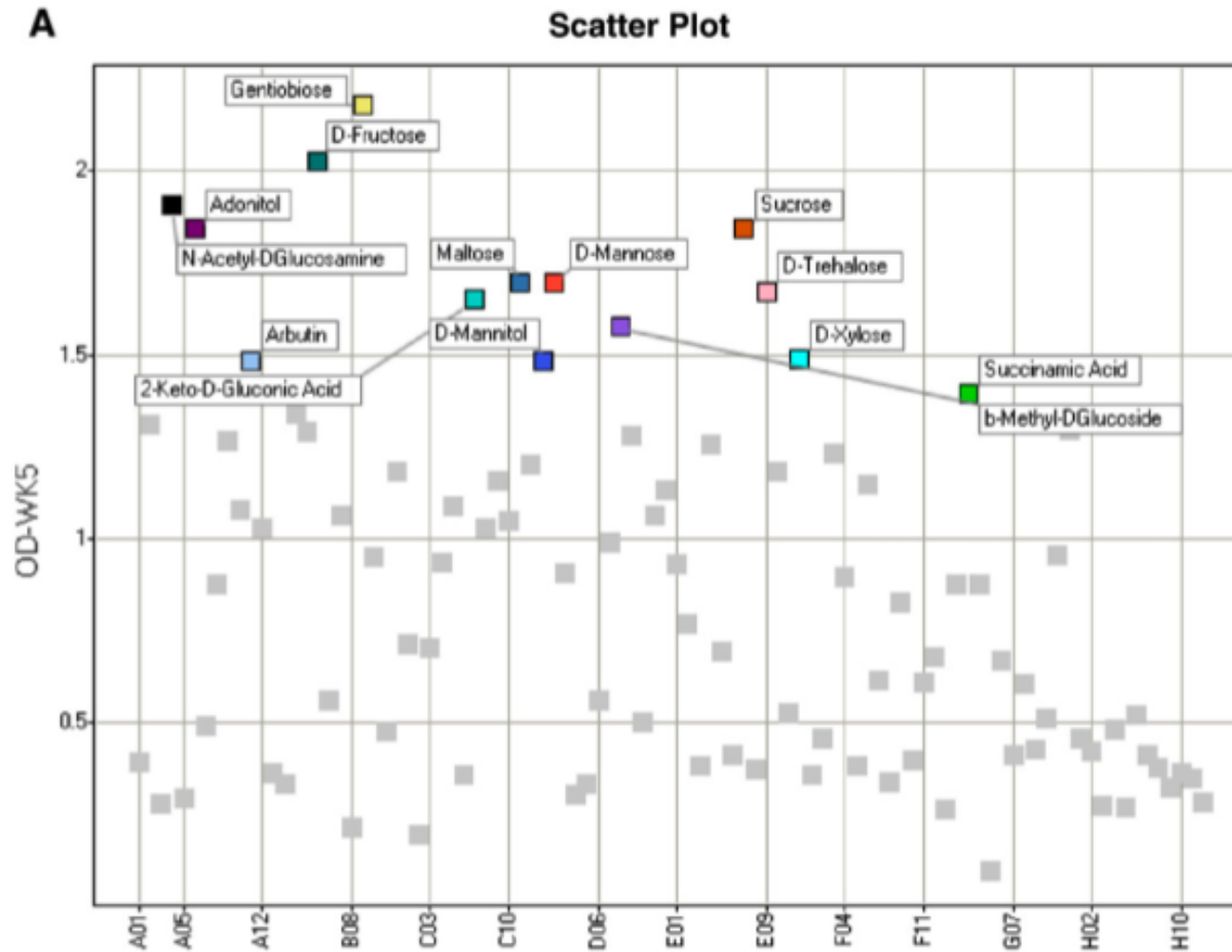
- Range of substrate utilization
- Growth
- Antimicrobial properties
- Secondary metabolite production
- Dereplication of closely related strains

M. Singh – J. Micro. Methods (2009) 77:102

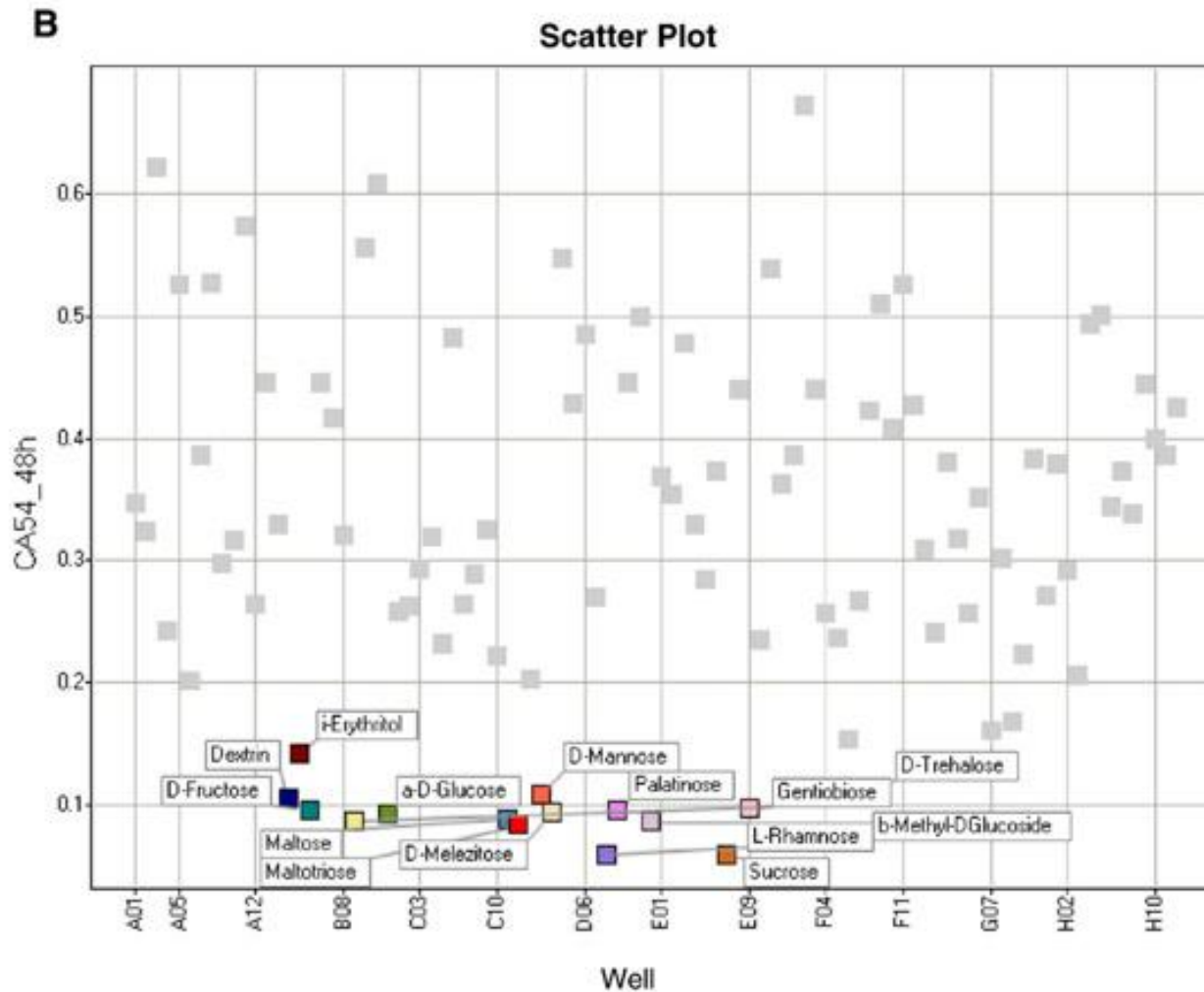


Scheme 1. Steps involved in the profiling of fungal cultures using FF MicroPlate.

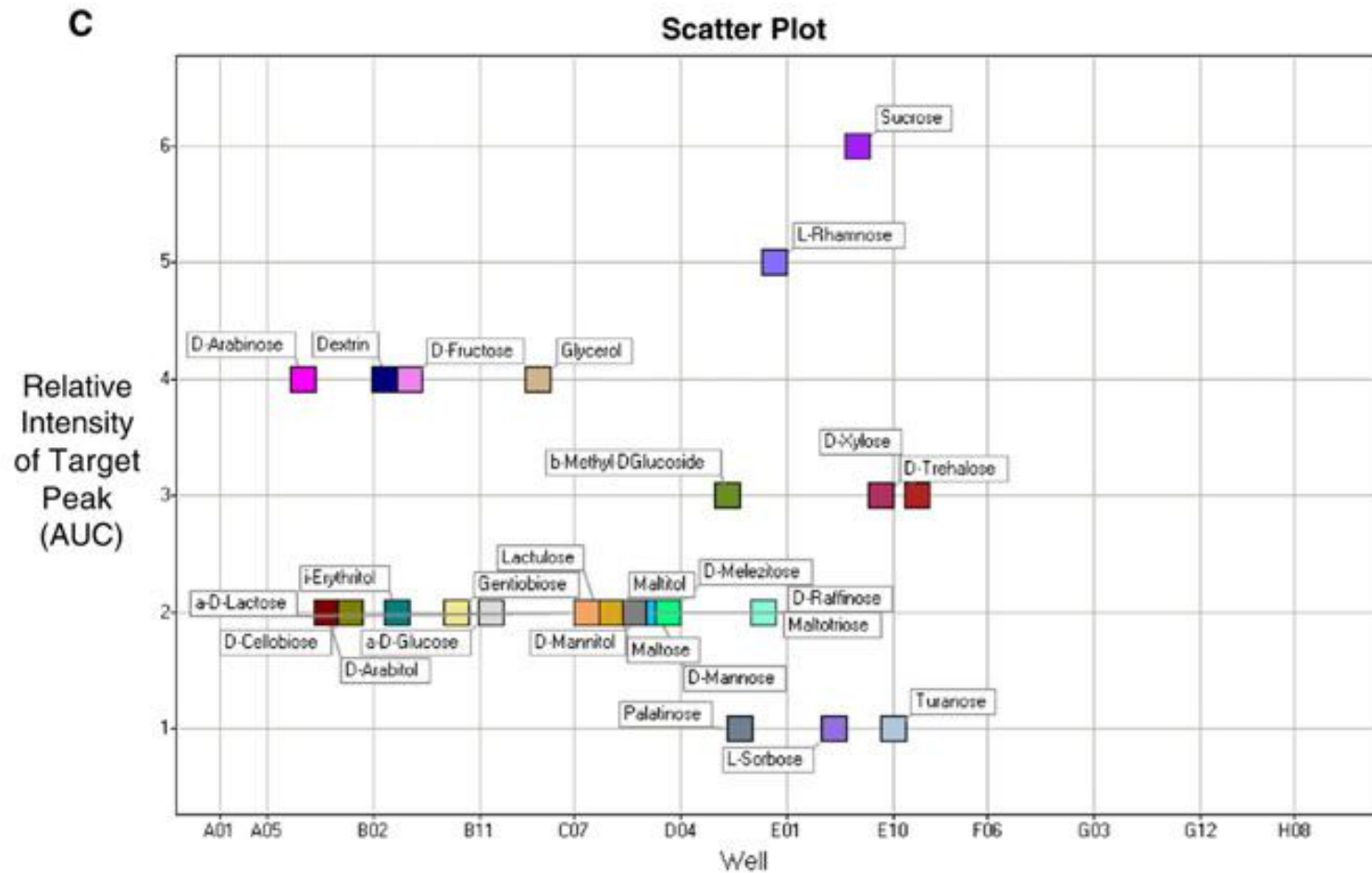
Substrate Utilization



Anti-microbial Activity

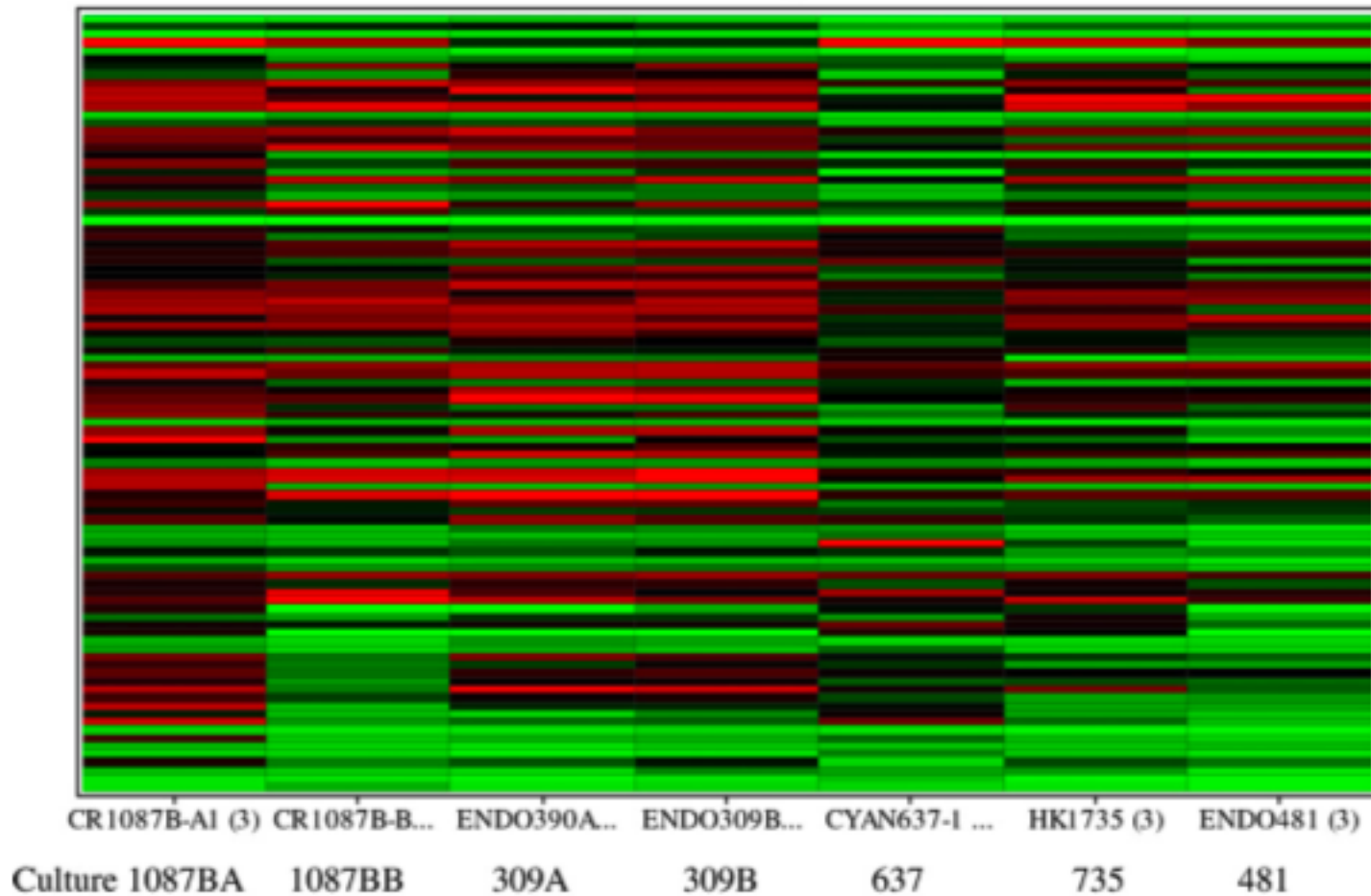


Target Compound Levels



Phomopsis spp. Dereplication

Heat Map



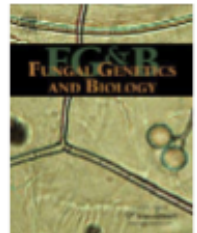
Induction of Toxin Synthesis in *Fusarium*



Contents lists available at [ScienceDirect](#)

Fungal Genetics and Biology

journal homepage: www.elsevier.com/locate/yfgbi

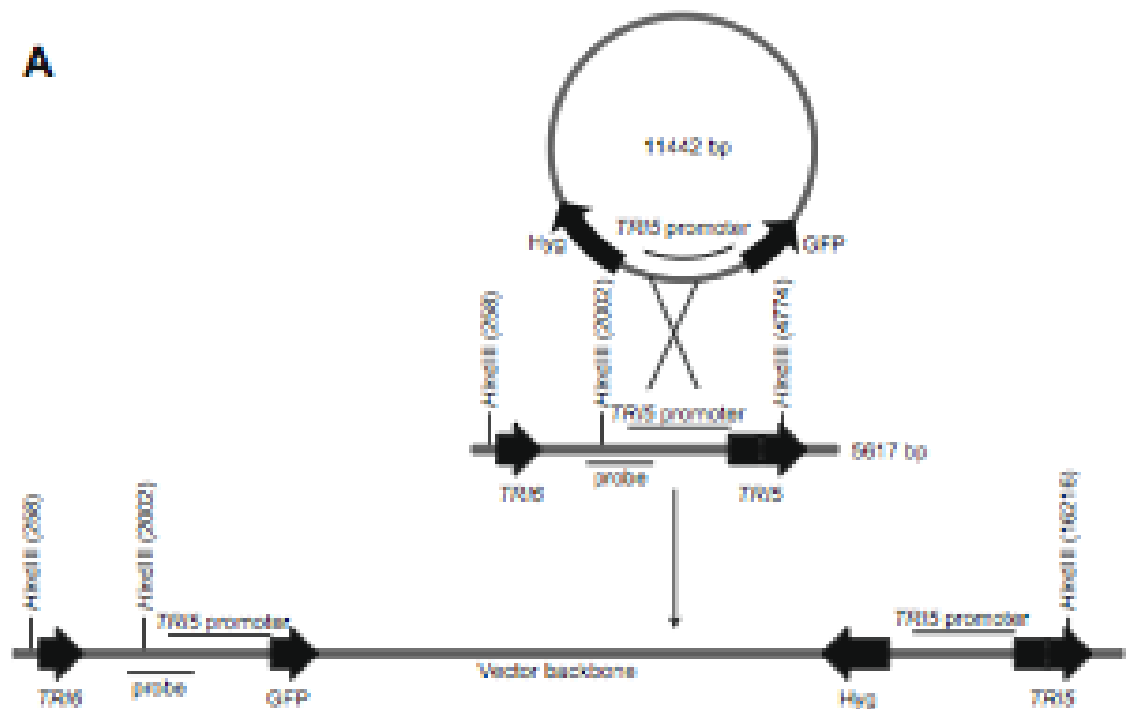


Nutrient profiling reveals potent inducers of trichothecene biosynthesis in *Fusarium graminearum*

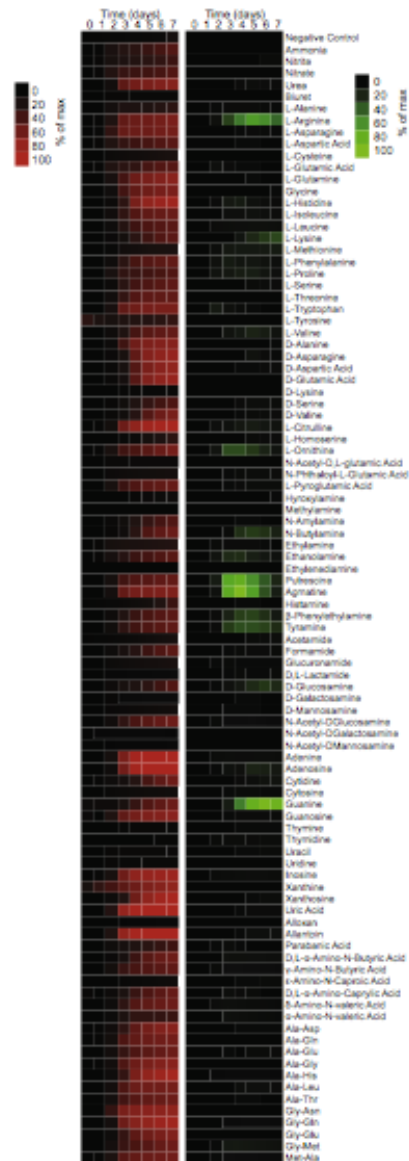
Donald M. Gardiner*, Kemal Kazan, John M. Manners

CSIRO Plant Industry, Queensland Bioscience Precinct, 306 Carmody Road, St. Lucia, Queensland 4067, Australia

Insertion of TRI5–GFP at the TRI5 locus



Culture Conditions Inducing Toxin Synthesis

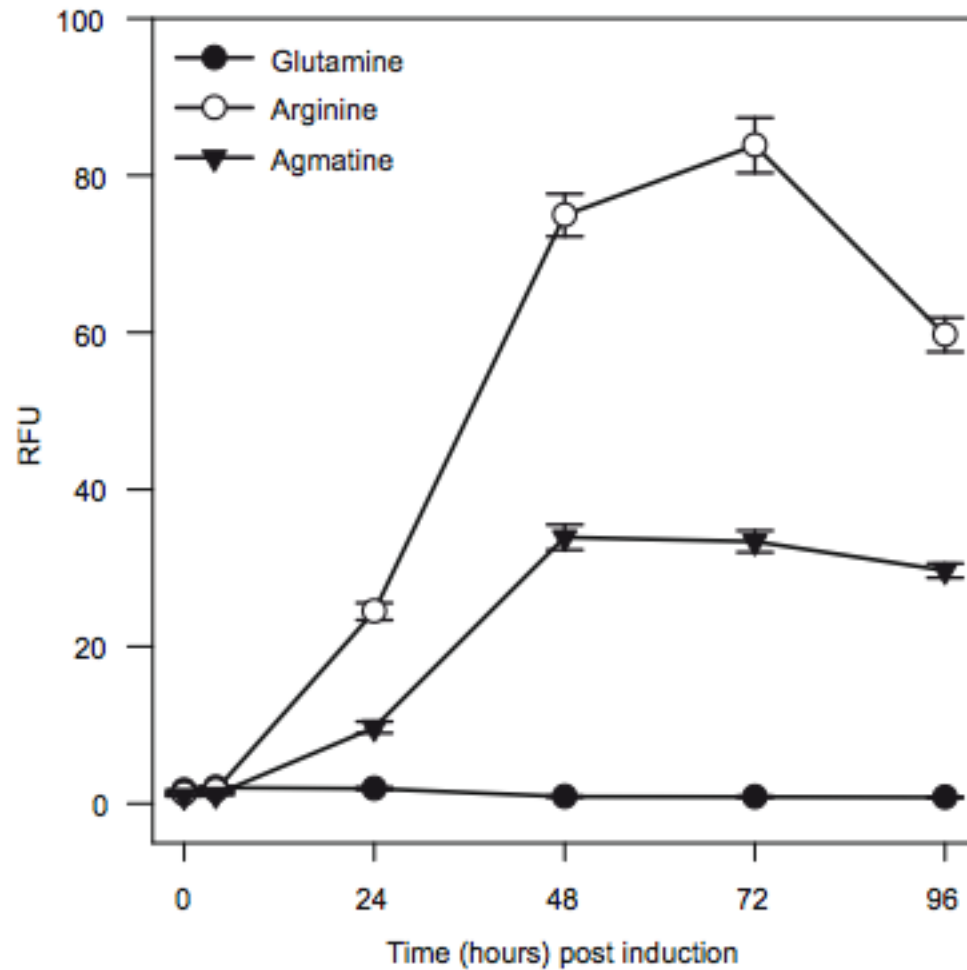


Culture conditions inducing synthesis of a trichothecene mycotoxin in the wheat pathogen, *Fusarium graminearum*.

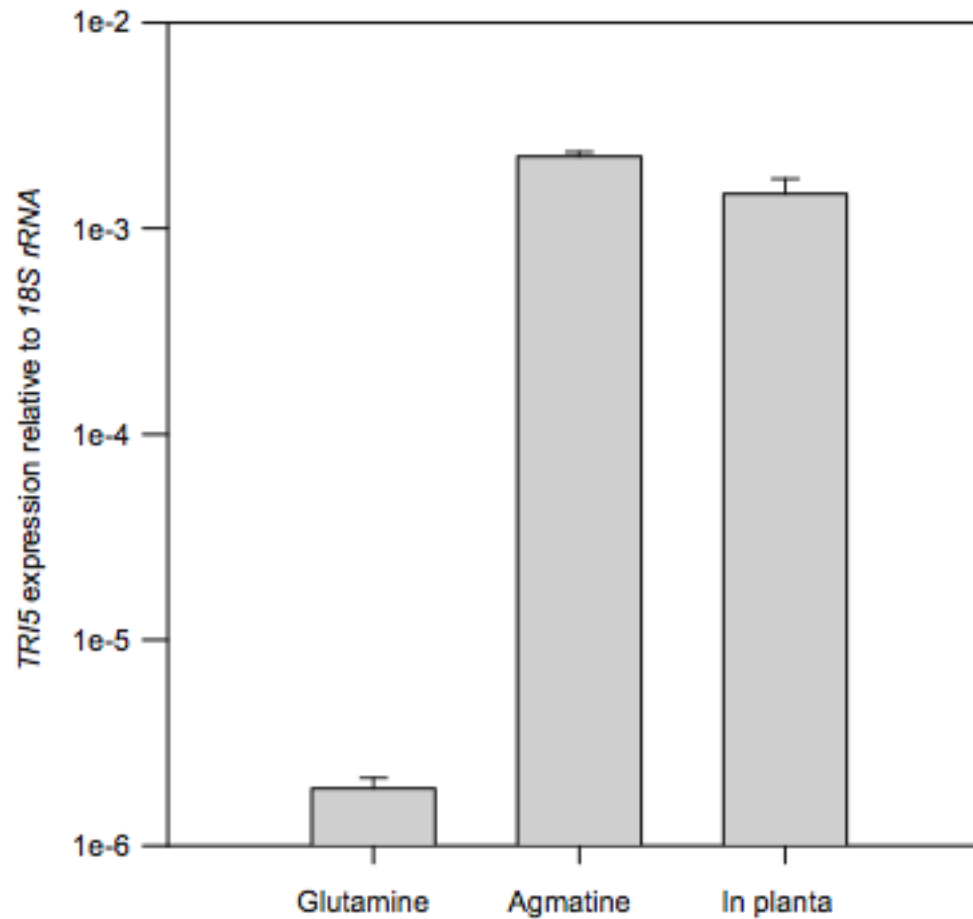
Induction was highest with arginine, putrescine, agmatine, and guanine as nitrogen sources.

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

Growth Independent Induction



In vitro vs. In planta



Carbon Source Dependent Sporulation

APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Jan. 2008, p. 245–250
0099-2240/08/\$08.00+0 doi:10.1128/AEM.02068-07
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Vol. 74, No. 1

Carbon Source Dependence and Photostimulation of Conidiation in *Hypocrea atroviridis*^{∇†}

Martina A. Friedl, Christian P. Kubicek, and Irina S. Druzhinina*

*Research Area of Gene Technology and Applied Biochemistry, Institute of Chemical Engineering, Vienna University of Technology,
Getreidemarkt 9-1665, A-1060 Vienna, Austria*

Received 11 September 2007/Accepted 22 October 2007

Carbon Source Dependent Sporulation

- *Hypocrea atroviridis* is frequently used as a photomorphogenetic model due to its ability to conidiate upon exposure to light. Light is thereby believed to be the primary trigger for spore formation.
- In contrast, we show here that conidiation is primarily carbon source dependent and that illumination plays a catalytic role;



Carbon Source Dependent Sporulation

- Of a total of 95 tested carbon sources, only a small set of carbohydrates, polyols, and sugar acids allowed conidiation in darkness, and on most of them, conidiation was significantly more strongly expressed in light.
- In addition, there are also a number of carbon sources on which *H. atroviridis* conidiates in darkness, but light does not further stimulate the process.
- Yet on another small set of carbon sources (L-sorbitol, D-fucose, D- and L-arabinose, and erythritol), *H. atroviridis* shows better sporulation in darkness than in light. No sporulation was observed on organic acids and amino acids.

Advantage of PM uses in Various Applications

Advantages:

- Robust and straightforward technology
- Automated incubation and data collection
- Complementary to genomic and proteomic technologies
- Bacterial, fungal and mammalian Cells

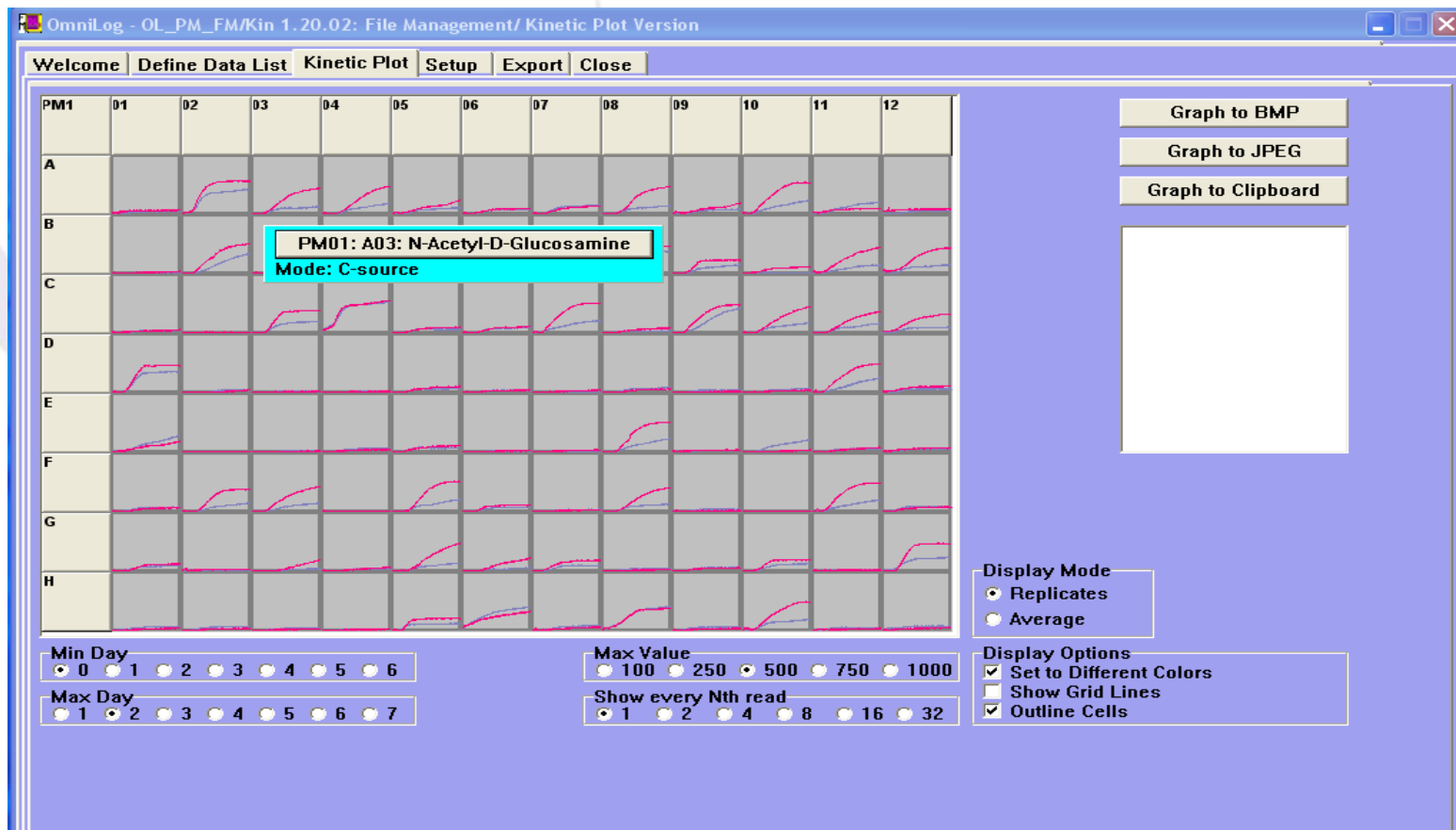
Applications:

- Quality assurance of stock or reference cultures
- Understanding metabolism in cells for basic research
- Functional genomics
- Inferring MOA of new drug compounds
- Pathogen host interactions
- Optimal conditions for growth, selection of specialized cell lines, development of selective assays



OmniLog PM Data Analysis

OmniLog PM Software

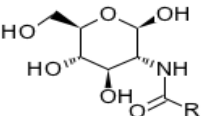


OmniLog PM Software



COMPOUND: C03000

Help

Entry	C03000	Compound
Name	N-Acyl-D-glucosamine	
Formula	C7H12NO6R	
Structure	 C03000 Mol file KCF file DB search	
Reaction	R02652	
Enzyme	5.1.3.8	
Other DBs	PubChem: 5909	
LinkDB	All DBs	
KCF data	Show	

=> [Original format](#)

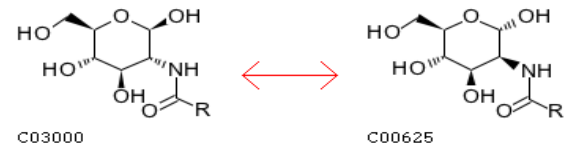
DBGET integrated database retrieval system, [GenomeNet](#)

OmniLog PM Software



REACTION: R02652

Help

Entry	R02652	Reaction
Name	N-Acyl-D-glucosamine 2-epimerase	
Definition	N-Acyl-D-glucosamine <=> N-Acyl-D-mannosamine	
Equation	C03000 <=> C00625	
	 C03000 C00625	
RPair	RP: A02383 C00625_C03000 main	
Enzyme	5.1.3.8	
LinkDB	All DBs	



REACTION: R01207

Help

Entry	R01207	Reaction
Name	N-Acyl-D-glucosamine 2-epimerase	
Definition	N-Acetyl-D-glucosamine <=> N-Acetyl-D-mannosamine	
Equation	C00140 <=> C00645	
	 C00140 C00645	
RPair	RP: A01362 C00140_C00645 main	
Pathway	PATH: rn00530 Aminosugars metabolism	
Enzyme	5.1.3.8	
Ortholog	KO: K01787 N-acylglucosamine 2-epimerase	
LinkDB	All DBs	

OmniLog PM Software



ENZYME: 5.1.3.8

Help

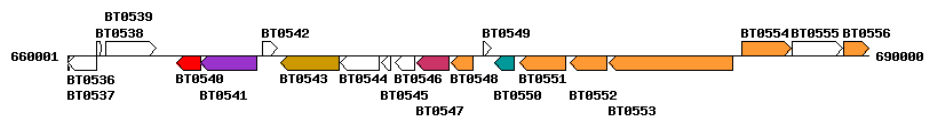
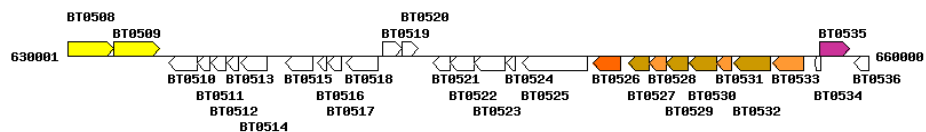
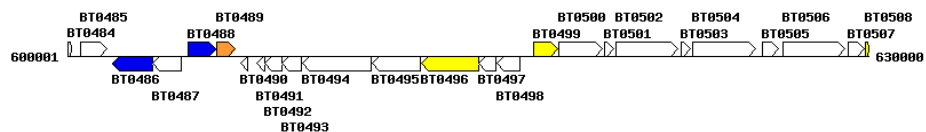
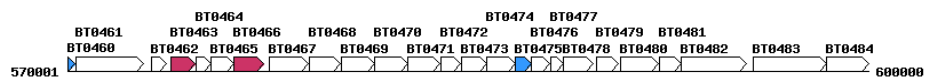
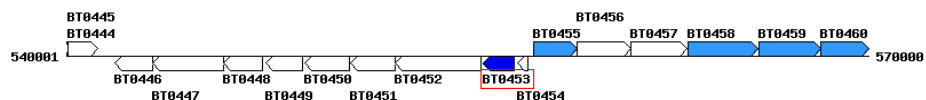
Entry	EC 5.1.3.8 Enzyme
Name	N-acylglucosamine 2-epimerase; acylglucosamine 2-epimerase; N-acetylglucosamine 2-epimerase
Class	Isomerases Racemases and epimerases Acting on carbohydrates and derivatives
Sysname	N-acyl-D-glucosamine 2-epimerase
Reaction (IUBMB)	N-acyl-D-glucosamine = N-acyl-D-mannosamine [RN:R02652]
Reaction (KEGG)	R02652 > R01207 Show all
Substrate	N-acyl-D-glucosamine [CPD:C03000]
Product	N-acyl-D-mannosamine [CPD:C00625]
Cofactor	ATP [CPD:C00002]
Comment	Requires catalytic amounts of ATP.
Pathway	PATH: map00530 Aminosugars metabolism
Ortholog	KO: K01787 N-acylglucosamine 2-epimerase
Genes	HSA: 5973 (RENBP) PTR: 465932 (LOC465932) MMU: 19703 (Renbp) RNO: 81759 (Renbp) SSC: 396934 (RENBP) BPM: BURPS1710b_0527 HNE: HNE_0032 HNE_0782 RBA: RB3348 SYN: slr1975 ANA: all3695 AVA: Ava_3567 BTH: BT0453
Disease	MIM: 312420 Renin-binding protein
Structures	PDB: 1FP3
Reference	1 Ghosh, S. and Roseman, S. The sialic acids. V. N-Acyl-D-glucosamine 2-epimerase. J. Biol. Chem. 240 (1965) 1531-1536.
Other DBs	IUBMB Enzyme Nomenclature: 5.1.3.8 ExPASy - ENZYME nomenclature database: 5.1.3.8

OmniLog PM Software

Entry	BT0453 CDS B.thetaiotaomicron
Definition	N-acylglucosamine 2-epimerase [EC:5.1.3.8]
KO	KO: K01787 N-acylglucosamine 2-epimerase
Pathway	PATH: bth00530 Aminosugars metabolism
Class	Gene catalog
SSDB	Ortholog Paralog Gene cluster
Motif	Pfam: GlcNAc_2-epim Motif
Other DBs	NCBI-GI: 29345863 NCBI-GeneID: 1071649 UniProt: Q8AAL1
LinkDB	PDB All DBs
Position	complement(555562..556725) Genome map
AA seq	387 aa AA seq DB search MDFKKLANQYRDELLDNVLPFWLEHSQDLEFGGYFSCLDREGKVFDTDKFIWLQGREVWM FSMLYNKVEKRQEWLDCAVQGGEFLKKYGHGDNYNWYFSLDRSGRPLVEPYNIFSITFAT MAFGQLSLATGNQEYADIAKTKFKIILSKVDNPKSKWNKLHPGTRNLKNFALPMILCNLA LEIEHLDPGYLEQTMETCIHEVMDVFYRPELGGIIIVENVMDGNLVDCFEGRQVTPGHA IEAMWFIMDLGKRLNRPKLIEKADVTLTMLDYGWDKQYGGIYYFMDRNGCPPQLEWDQ KLWWVHIESLISLLKGYQLTGDRKCLEWFEKVHDYTWSHFKDPEYFEWGYLNRGEVLL PLKGGKWKGCFFHVRGLFQCWKVLEPL
NT seq	1164 nt NT seq +upstream0 nt +downstream0 nt atggattttaagaaaactagcgaatcagtagcgggatgaattgttggaacatgtccttcca ttctggctogagcactctcaagatcttgagtttggcgggtatttccagctgcoctggacogt gaaggggaaggttttgcatacggataagttcatctgggtgcagggacgtgaggtatggatg ttctccatgctttacaacaaagtggaaaaacgacaggaatggctggattgtgcocttcag ggtggtgaattcttaaaaaatacggacatgatggaattataactggtacttttcactc gacogtctcgggttagaccactggtagaacacatacaaatattttctcatatactttcgccaag atggccttcggacagctgagtttagcaactggttaatacaggaatatgcggacatagcaaaag aagactttcaagatcatcctttccaaggtgataatccgaaaagcaagtggaacaagctt catccgggtactcgtaatctgaagaacttcgcccctccgatgatcctctgtaactctggt ttggagatcgaaacatctcctggatcccggtatctctggaacagacgatggaacacgtgta catgaagtgatggatgtcttttatcgtccggagctgggggcatcattgttgagaatgtc gatatggacggtaactctggtgattgcttcgaaggtcgtcaggtgactcccggtcactgct atcgaagcgatgtggtttatcatggatctgggcaaacgcctgaatcgtccgaaattgata gaaaaagcaaaagacgtaaacctgacaatgctcgactatggttgggacaagcaatatgga ggtatctattactttatggacgttaacggtttgtcctccccagcaattggaatgggatcag aaacttttggtgggtgcatattgaatctctgatatactgctgaaaggttatcagctgacc ggagacaggaaatgtctggaatggtttgaaaaagtgcatgactacacatgggtgcacttc aaagatccggaatatccggaatggtacggatactgaatcgacagaggggaagttttgta ccgctgaaaaggtggttaattggaaggtgtcttcatgtaccaaggggattatccaatgc tggaagattagaaacgcgtatag

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	Carbohydrate Metabolism		Transcription
	Energy Metabolism		Translation
	Lipid Metabolism		Folding, Sorting and Degradation
	Nucleotide Metabolism		Replication and Repair
	Amino Acid Metabolism		Membrane Transport
	Metabolism of Other Amino Acids		Signal Transduction
	Glycan Biosynthesis and Metabolism		Signaling Molecules and Interaction
	Biosynthesis of Polyketides and Nonribosomal Peptides		Cellular Processes
	Metabolism of Cofactors and Vitamins		Unassigned
	Xenobiotics Biodegradation and Metabolism		Not found in catalog

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