INSTRUCTIONS FOR USE

(PM Kit, Biolog Catalog # 12191)
(PM1 MicroArray, Biolog Catalog # 12111)
(PM2 MicroArray, Biolog Catalog # 12112)
(PM3 MicroArray, Biolog Catalog # 12121)
(PM4 MicroArray, Biolog Catalog # 12131)

PHENOTYPE MICROARRAY™ TECHNOLOGY

INTRODUCTION AND DESCRIPTION

A key aspect of most biological research and development involves measuring the traits or phenotypes of cells. Biolog, Inc. has developed a new proprietary technology called Phenotype MicroArrays (PMs). PMs allow biologists, for the first time, to globally analyze the phenotypes of cells. Thousands of phenotypes can be tested simultaneously in a very simple, efficient, high-throughput standardized format. The current PM technology is developed for use with diverse microbial cells including fungi as well as bacteria.

PMs are sets of 96-well microtiter plates with each well containing a different cell culture medium that is designed to test a unique phenotype or cell function. The components of the culture media are dried onto the bottom of each well. To run a large number of tests, the user has only to add a cell suspension. After inoculation the cells are incubated, typically for 24 to 48 hours, and their phenotypic behavior is observed and recorded.

The response of the cells in each well is monitored colorimetrically using a patented redox chemistry that integrates the respiration of the cells over the time course of the incubation. The result is a color change, for example, from colorless to purple. If the cells display an expected phenotype and can grow in a well, they respire normally to give a dark purple color. If the phenotype and growth are weak, they respire more slowly and give a light purple color. If the phenotype and growth are negative, the well remains colorless.

OVERVIEW OF THE PM TEST PROCEDURE

Phenotype MicroArrays [1-4] measure the carbon (PM1-2), nitrogen (PM3), and phosphorus/sulfur (PM4) metabolism of a strain. Standardized cell suspensions are prepared and inoculated into the PMs. As the PMs are incubated under appropriate conditions, color forms in the wells to reflect the phenotypes of the strain being tested.

The procedure outlined in this document has been optimized for *E. coli*, *Salmonella spp.*, and other enteric bacteria. It can also be applied to other microbes, but the protocol may require minor modification.

The basic steps are described below and Table 1 summarizes the modifications required for exemplary species. The modifications are listed as the columns in Table 1: medium for cultivation, incubation temperature, incubation atmosphere, inoculum cell density, inoculum volume per well, additions to the IF-0 inoculating fluid for PM1-2, and additions to the inoculating fluid for PM3-4.

Before getting started you need to have ready a means for preparing and inoculating cell suspensions into the PMs. Also, you need to know whether your strain requires special organic or inorganic growth supplements. If it does, these must be provided as additional (auxotrophic) supplements in the IF-0 inoculating fluids for PM3 and PM4.

MATERIALS

The following materials are either helpful or needed in the procedure (some chemicals prepared as sterile solutions may also be required as specified in Table 1):

Step	*Description*	*Catalog#*	*Source*
1	R2A Agar Plates	Bio-M1065	Biolog
1	BUG+B Agar Plates	71102	Biolog
1	R2A Agar Powder	7390B	Acumedia[1]
1	BUG Agar Powder	70101	Biolog
2	Biolog Turbidimeter	3531	Biolog
2	85%T Turbidity Standard	3431	Biolog
2	40%T Turbidity Standard	3432	Biolog
2	IF-0 Inoculating Fluid	72211	Biolog
2	LongSwabs	3021	Biolog
3	Electronic Multichannel Pipetter	3501A	Biolog
3	Sterile Pipette Tips	3001	Biolog
3	Sterile Filling Reservoirs	3002	Biolog

[1]Acumedia is a subsidiary of Neogen, Corp. (East Lansing, MI)

TEST PROCEDURE - 5 BASIC STEPS

STEP 1: Culture the Organism. Culture the organism overnight on R2A Agar or BUG+B Agar as specified in Table 1. R2A Agar is a rich medium with low nutrient concentrations [5]. For many species this medium helps by depleting stores of phosphorus and sulfur from the cells, lowering the background respiration in the PM4 MicroArray. Some strains do not grow well or give poor results when cultured on R2A. These strains are grown on BUG+B Agar instead. After streaking the plates, incubate them overnight following the temperature and atmosphere recommendations in Table 1.

STEP 2: Prepare Cell Suspensions. First determine the target cell density. This is done with turbidity standards available from Biolog (e.g. 85%T or 40%T). Either use a turbidimeter or spectrophotometer that will accommodate a 20 mm glass tube (e.g. the Biolog Turbidimeter), or open the turbidity standard, pour it into the appropriate cuvette, and determine the target reading on your photometer.

To suspend the cells in the special inoculating fluid provided (IF-0, perhaps with supplements) use a dry sterile cotton swab to remove a small amount of cells from the agar surface. Rub the swab with cells against the inner wall of the glass tube above the meniscus using a circular motion to crush any clumps or mucus, then gradually slide the cells up and down into the inoculating fluid. Be sure to eliminate all clumps. Stir the cell suspension with the swab to homogenize the suspension.

Avoid turbulent vortexing which can trap air bubbles in the viscous inoculating fluid. Adjust the turbidity to correspond precisely with the target cell density. This is very important! If the turbidity is too light, add more cells. If it is too dense, dilute it with inoculating fluid.

STEP 3: Inoculate a Cell Suspension into each PM. Pour each cell suspension into a sterile filling reservoir and inoculate the PMs by pipetting 100 µL per well.

STEP 4: Incubate the PMs. Incubate the PMs at the recommended temperature (Table 1) in a humidified incubator to prevent drying of the outer wells. PM1 and PM2 are typically incubated for 24 hr and PM3 and PM4 for 36-48 hr.

STEP 5: Read and Score the Results. Examine the wells for the formation of purple color which indicates utilization of the C, N, P, or S source in a particular well. Use the A-1 well as a reference well for a "negative" reaction (also the F-1 well in PM4). Any well with more color than the reference well is considered "positive". Typical reactions in PM1-4 for the species listed in Table 1 are posted on Biolog's website (www.biolog.com). Different strains will vary, but your result with non-mutated strains should be similar.

PROTOCOLS FOR SPECIFIC SPECIES

Recommended testing protocols are summarized in Table 1 below. PM1-2 measure carbon utilization in a fully supplemented medium and should not require any supplements to the inoculating fluid other than menadione, salicylate or thioglycolate. PM3-4 measure nitrogen, phosphorus, and sulfur utilization in a minimal defined medium. A carbon source must always be added to the inoculating fluid for these PMs. The choice of the carbon source can have a strong effect on the result obtained. For example, succinate is recommended instead of glucose for enteric bacteria because many more positive reactions are obtained (glucose represses formation of many secondary utilization pathways).

The supplements in Table 1 have been optimized and they should be prepared and added precisely as specified unless there are reasons to depart from the recommendations. Also, for PM3-4, you need to know whether your strain requires special organic or inorganic growth supplements to be added to the minimal inoculating fluid. If it does, these must be provided as additional supplements in the inoculating fluids for PM3 and PM4.

ADDITIONAL TECHNICAL ASSISTANCE AND WEBSITE INFORMATION

The most current information on Phenotype MicroArrays is posted on the Biolog website (www.biolog.com). If you need additional help or advice, please call us before you attempt to use the Phenotype MicroArrays. You can call Biolog Technical Service at 510-785-2564 or send an Email to info@biolog.com.

REFERENCES

[1] Phenotype MicroArrays for High Throughput Phenotypic Testing and Assay of Gene Function. B.R. Bochner, P. Gadzinski, and E. Panomitros, Genome Research, 2001, v. 11, p. 1246-1255.

[2] Sleuthing Out Bacterial Identities. B.R. Bochner, Nature, 1989, v.339, p.157-158.

[3] The Consequences of Growth of a Mutator Strain of *Escherichia coli* as Measured by Loss of Function Among Multiple Gene Targets and Loss of Fitness. P. Funchain, A. Yeung, J.L. Stewart, R. Lin, M.M. Slupska, and J.H. Miller, Genetics, 2000, v. 154, p. 959-970.

[4] Megaplasmid pRme2011a of *Sinorhizobium meliloti* Is Not Required for Viability. I.J. Oresnik, S.-L. Liu, C.K. Yost, and M.F. Hines, Journal of Bacteriology, 2000, v. 182, p.3582-3586.

[5] A new medium for the enumeration and subculture of bacteria from potable water. D.J. Reasoner and E.E. Geldreich, Applied and Environmental Microbiology, 1985, v. 49, p. 1-7.

TABLE 1

Species	Medium	Temp	Atm	Density	Inoculum	* Add to IF-0 PM1-2	PM3-4
Gram Negative Bacteria							
Escherichia coli	R2A	35-37	Air	85%T	100µL		B
Salmonella typhimurium	R2A	35-37	Air	85%T	100µL		B
Pseudomonas aeruginosa	R2A	35-37	Air	85%T	100µL	A	C
Burkholderia cepacia	R2A	30	Air	85%T	100µL		B
Ralstonia pickettii	R2A	30	Air	85%T	100µL		B
Ralstonia solanacearum	R2A	30	Air	85%T	100µL		B
Vibrio fluvialis	R2A	30	Air	85%T	100µL		B
Sinorhizobium meliloti	R2A	30	Air	85%T	100µL		B
Gram Positive Bacteria							
Listeria monocytogenes	BUG+B	35-37	Air	40%T	100µL	D	E

* Additions to IF-0: Prepare a 100x stock solution as described below. Dissolve the chemicals in 10 ml of purified water, filter sterilize, and store in a refrigerator wrapped in aluminum foil (some of the chemicals are light-sensitive). Add 150 µL of the 100x stock solution to 15 ml of IF-0 inoculating fluid before preparing the cell suspensions. All chemicals are available from Sigma Chemical Company (St. Louis, MO). The Sigma catalog numbers are indicated below.

A: menadione sodium bisulfite (Sigma Cat# M5750) 5.524 mg

B: sodium succinate (Sigma Cat# S2378) 5.402 g + ferric citrate (Sigma Cat# F6129) 0.49 mg

C: sodium succinate (Sigma Cat# S2378) 5.402 g + ferric citrate (Sigma Cat# F6129) 0.49 mg + menadione sodium bisulfite (Sigma Cat# M5750) 5.524 mg - menadione is added for *Pseudomonas aeruginosa* to inhibit formation of the green pyocyanin pigment which interferes with the colorimetric assay

D: menadione sodium bisulfite (Sigma Cat# M5750) 5.524 mg + sodium salicylate (Sigma Cat# S3007) 320.2 mg

E: sodium pyruvate (Sigma Cat# P2256) 2.2 g + sodium thioglycolate (Sigma Cat# T0632) 570.5 mg + uridine (Sigma Cat# U3750) 24.42 mg

PM1 MicroPlate™

A1 Negative Control	A2 L-Arabinose	A3 N-Acetyl-D-Glucosamine	A4 D-Saccharic Acid	A5 Succinic Acid	A6 D-Galactose	A7 L-Aspartic Acid	A8 L-Proline	A9 D-Alanine	A10 D-Trehalose	A11 D-Mannose	A12 Dulcitol
B1 D-Serine	B2 D-Sorbitol	B3 Glycerol	B4 L-Fucose	B5 D-Glucuronic Acid	B6 D-Gluconic Acid	B7 D,L-α-Glycerol-Phosphate	B8 D-Xylose	B9 L-Lactic Acid	B10 Formic Acid	B11 D-Mannitol	B12 L-Glutamic Acid
C1 Glucose-6-Phosphate	C2 D-Galactonic Acid-γ-Lactone	C3 D,L-Malic Acid	C4 D-Ribose	C5 Tween 20	C6 L-Rhamnose	C7 D-Fructose	C8 Acetic Acid	C9 α-D-Glucose	C10 Maltose	C11 D-Melibiose	C12 Thymidine
D-1 L-Asparagine	D2 D-Aspartic Acid	D3 D-Glucosaminic Acid	D4 1,2-Propanediol	D5 Tween 40	D6 α-Keto-Glutaric Acid	D7 α-Keto-Butyric Acid	D8 α-Methyl-D-Galactoside	D9 α-D-Lactose	D10 Lactulose	D11 Sucrose	D12 Uridine
E1 L-Glutamine	E2 M-Tartaric Acid	E3 Glucose-1-Phosphate	E4 Fructose-6-Phosphate	E5 Tween 80	E6 α-Hydroxy Glutaric Acid-γ-Lactone	E7 α-Hydroxy Butyric Acid	E8 β-Methyl-D-Glucoside	E9 Adonitol	E10 Maltotriose	E11 2-Deoxy Adenosine	E12 Adenosine
F1 Glycyl-L-Aspartic Acid	F2 Citric Acid	F3 M-Inositol	F4 D-Threonine	F5 Fumaric Acid	F6 Bromo Succinic Acid	F7 Propionic Acid	F8 Mucic Acid	F9 Glycolic Acid	F10 Glyoxylic Acid	F11 D-Cellobiose	F12 Inosine
G1 Glycyl-L-Glutamic Acid	G2 Tricarballylic Acid	G3 L-Serine	G4 L-Threonine	G5 L-Alanine	G6 L-Alanyl-Glycine	G7 Acetoacetic Acid	G8 N-Acetyl-β-D-Mannosamine	G9 Mono Methyl Succinate	G10 Methyl Pyruvate	G11 D-Malic Acid	G12 L-Malic Acid
H1 Glycyl-L-Proline	H2 P-Hydroxy Phenyl Acetic Acid	H3 M-Hydroxy Phenyl Acetic Acid	H4 Tyramine	H5 D-Psicose	H6 L-Lyxose	H7 Glucuronamide	H8 Pyruvic Acid	H9 L-Galactonic Acid-γ-Lactone	H10 D-Galacturonic Acid	H11 Phenylethyl-amine	H12 2-Aminoethanol

FIGURE 1. Carbon Sources in PM1 MicroArray

PM2A MicroPlate™

A1 Negative Control	A2 Chondroitin Sulfate C	A3 α-Cyclodextrin	A4 β-Cyclodextrin	A5 γ-Cyclodextrin	A6 Dextrin	A7 Gelatin	A8 Glycogen	A9 Inulin	A10 Laminarin	A11 Mannan	A12 Pectin
B1 N-Acetyl-D-Galactosamine	B2 N-Acetyl-Neuraminic Acid	B3 β-D-Allose	B4 Amygdalin	B5 D-Arabinose	B6 D-Arabitol	B7 L-Arabitol	B8 Arbutin	B9 2-Deoxy-D-Ribose	B10 I-Erythritol	B11 D-Fucose	B12 3-0-β-D-Galacto-pyranosyl-D-Arabinose
C1 Gentiobiose	C2 L-Glucose	C3 Lactitol	C4 D-Melezitose	C5 Maltitol	C6 α-Methyl-D-Glucoside	C7 β-Methyl-D-Galactoside	C8 3-Methyl Glucose	C9 β-Methyl-D-Glucuronic Acid	C10 α-Methyl-D-Mannoside	C11 β-Methyl-D-Xyloside	C12 Palatinose
D1 D-Raffinose	D2 Salicin	D3 Sedoheptulosan	D4 L-Sorbose	D5 Stachyose	D6 D-Tagatose	D7 Turanose	D8 Xylitol	D9 N-Acetyl-D-Glucosaminitol	D10 γ-Amino Butyric Acid	D11 δ-Amino Valeric Acid	D12 Butyric Acid
E1 Capric Acid	E2 Caproic Acid	E3 Citraconic Acid	E4 Citramalic Acid	E5 D-Glucosamine	E6 2-Hydroxy Benzoic Acid	E7 4-Hydroxy Benzoic Acid	E8 β-Hydroxy Butyric Acid	E9 γ-Hydroxy Butyric Acid	E10 α-Keto Valeric Acid	E11 Itaconic Acid	E12 5-Keto-D-Gluconic Acid
F1 D-Lactic Acid Methyl Ester	F2 Malonic Acid	F3 Melibionic Acid	F4 Oxalic Acid	F5 Oxalomalic Acid	F6 Quinic Acid	F7 D-Ribono-1,4-Lactone	F8 Sebacic Acid	F9 Sorbic Acid	F10 Succinamic Acid	F11 D-Tartaric Acid	F12 L-Tartaric Acid
G1 Acetamide	G2 L-Alaninamide	G3 N-Acetyl-L-Glutamic Acid	G4 L-Arginine	G5 Glycine	G6 L-Histidine	G7 L-Homoserine	G8 Hydroxy-L-Proline	G9 L-Isoleucine	G10 L-Leucine	G11 L-Lysine	G12 L-Methionine
H1 L-Ornithine	H2 L-Phenylalanine	H3 L-Pyroglutamic Acid	H4 L-Valine	H5 D,L-Carnitine	H6 Sec-Butylamine	H7 D.L-Octopamine	H8 Putrescine	H9 Dihydroxy Acetone	H10 2,3-Butanediol	H11 2,3-Butanone	H12 3-Hydroxy 2-Butanone

FIGURE 2. Carbon Sources in PM2 MicroArray

PM3B MicroPlate™

A1 Negative Control	A2 Ammonia	A3 Nitrite	A4 Nitrate	A5 Urea	A6 Biuret	A7 L-Alanine	A8 L-Arginine	A9 L-Asparagine	A10 L-Aspartic Acid	A11 L-Cysteine	A12 L-Glutamic Acid
B1 L-Glutamine	B2 Glycine	B3 L-Histidine	B4 L-Isoleucine	B5 L-Leucine	B6 L-Lysine	B7 L-Methionine	B8 L-Phenylalanine	B9 L-Proline	B10 L-Serine	B11 L-Threonine	B12 L-Tryptophan
C1 L-Tyrosine	C2 L-Valine	C3 D-Alanine	C4 D-Asparagine	C5 D-Aspartic Acid	C6 D-Glutamic Acid	C7 D-Lysine	C8 D-Serine	C9 D-Valine	C10 L-Citrulline	C11 L-Homoserine	C12 L-Ornithine
D-1 N-Acetyl-D,L-Glutamic Acid	D2 N-Phthaloyl-L-Glutamic Acid	D3 L-Pyroglutamic Acid	D4 Hydroxylamine	D5 Methylamine	D6 N-Amylamine	D7 N-Butylamine	D8 Ethylamine	D9 Ethanolamine	D10 Ethylenediamine	D11 Putrescine	D12 Agmatine
E1 Histamine	E2 β-Phenylethyl-amine	E3 Tyramine	E4 Acetamide	E5 Formamide	E6 Glucuronamide	E7 D,L-Lactamide	E8 D-Glucosamine	E9 D-Galactosamine	E10 D-Mannosamine	E11 N-Acetyl-D-Glucosamine	E12 N-Acetyl-D-Galactosamine
F1 N-Acetyl-D-Mannosamine	F2 Adenine	F3 Adenosine	F4 Cytidine	F5 Cytosine	F6 Guanine	F7 Guanosine	F8 Thymine	F9 Thymidine	F10 Uracil	F11 Uridine	F12 Inosine
G1 Xanthine	G2 Xanthosine	G3 Uric Acid	G4 Alloxan	G5 Allantoin	G6 Parabanic Acid	G7 D,L-α-Amino-N-Butyric Acid	G8 γ-Amino-N-Butyric Acid	G9 ε-Amino-N-Caproic Acid	G10 D,L-α-Amino-Caprylic Acid	G11 δ-Amino-N-Valeric Acid	G12 α-Amino-N-Valeric Acid
H1 Ala-Asp	H2 Ala-Gln	H3 Ala-Glu	H4 Ala-Gly	H5 Ala-His	H6 Ala-Leu	H7 Ala-Thr	H8 Gly-Asn	H9 Gly-Gln	H10 Gly-Glu	H11 Gly-Met	H12 Met-Ala

FIGURE 3. Nitrogen Sources in PM3 MicroArray

PM4A MicroPlate™

A1 Negative Control	A2 Phosphate	A3 Pyrophosphate	A4 Trimeta-phosphate	A5 Tripoly-phosphate	A6 Triethyl Phosphate	A7 Hypophosphite	A8 Adenosine- 2'-monophosphate	A9 Adenosine- 3'-monophosphate	A10 Adenosine- 5'-monophosphate	A11 Adenosine- 2',3'-cyclic monophosphate	A12 Adenosine- 3',5'-cyclic monophosphate
B1 Thiophosphate	B2 Dithiophosphate	B3 D,L-α-Glycerol Phosphate	B4 β-Glycerol Phosphate	B5 Carbamyl Phosphate	B6 D-2-Phospho-Glyceric Acid	B7 D-3-Phospho-Glyceric Acid	B8 Guanosine- 2'-monophosphate	B9 Guanosine- 3'-monophosphate	B10 Guanosine- 5'-monophosphate	B11 Guanosine- 2',3'-cyclic monophosphate	B12 Guanosine- 3',5'-cyclic monophosphate
C1 Phosphoenol Pyruvate	C2 Phospho-Glycolic Acid	C3 D-Glucose-1-Phosphate	C4 D-Glucose-6-Phosphate	C5 2-Deoxy-D-Glucose 6-Phosphate	C6 D-Glucosamine-6-Phosphate	C7 6-Phospho-Gluconic Acid	C8 Cytidine- 2'-monophosphate	C9 Cytidine- 3'-monophosphate	C10 Cytidine- 5'-monophosphate	C11 Cytidine- 2',3'-cyclic monophosphate	C12 Cytidine- 3',5'-cyclic monophosphate
D1 D-Mannose-1-Phosphate	D2 D-Mannose-6-Phosphate	D3 Cysteamine-S-Phosphate	D4 Phospho-L-Arginine	D5 O-Phospho-D-Serine	D6 O-Phospho-L-Serine	D7 O-Phospho-L-Threonine	D8 Uridine- 2'-monophosphate	D9 Uridine- 3'-monophosphate	D10 Uridine- 5'-monophosphate	D11 Uridine- 2',3'-cyclic monophosphate	D12 Uridine- 3',5'-cyclic monophosphate
E1 O-Phospho-D-Tyrosine	E2 O-Phospho-L-Tyrosine	E3 Phosphocreatine	E4 Phosphoryl Choline	E5 O-Phosphoryl-Ethanolamine	E6 Phosphono Acetic Acid	E7 2-Aminoethyl Phosphonic Acid	E8 Methylene Diphosphonic Acid	E9 Thymidine- 3'-monophosphate	E10 Thymidine- 5'-monophosphate	E11 Inositol Hexaphosphate	E12 Thymidine 3',5'-cyclic monophosphate
F1 Negative Control	F2 Sulfate	F3 Thiosulfate	F4 Tetrathionate	F5 Thiophosphate	F6 Dithiophosphate	F7 L-Cysteine	F8 D-Cysteine	F9 L-Cysteinyl-Glycine	F10 L-Cysteic Acid	F11 Cysteamine	F12 L-Cysteine Sulfinic Acid
G1 N-Acetyl-L-Cysteine	G2 S-Methyl-L-Cysteine	G3 Cystathionine	G4 Lanthionine	G5 Glutathione	G6 D,L-Ethionine	G7 L-Methionine	G8 D-Methionine	G9 Glycyl-L-Methionine	G10 N-Acetyl-D,L-Methionine	G11 L- Methionine Sulfoxide	G12 L-Methionine Sulfone
H1 L-Djenkolic Acid	H2 Thiourea	H3 1-Thio-β-D-Glucose	H4 D,L-Lipoamide	H5 Taurocholic Acid	H6 Taurine	H7 Hypotaurine	H8 p-Amino Benzene Sulfonic Acid	H9 Butane Sulfonic Acid	H10 2-Hydroxyethane Sulfonic Acid	H11 Methane Sulfonic Acid	H12 Tetramethylene Sulfone

FIGURE 4 Phosphorus & Sulfur Sources in PM4 MicroArray