Table 2. Summary of environments and algorithm results. 54 environments were identified that support growth on Biolog platesbut that can not be reconciled with the current metabolic reconstruction. All Biolog data comes from the company website(www.biolog.com/accesstoPMTech.html) with the exception of propionate (PM1 F7) where evidence from mutant growth on Biolog plates (availablefrom ASAP database) was used to indicate that *E. coli* could grow on propionate. The 54 environments are divided up into 5 cases: (1) cases where thealgorithm could identify solutions, (2) cases where no solutions could be found either because the metabolite didn't exist in the U matrix or none of thereactions in the matrix could explain growth, (3) environments with no matching KEGG ids (so they are not in the U matrix either), (4) known incorrectBiolog results, and (5) cases which could be reconciled with the current *E. coli* literature. For the 26 cases for which the algorithm could identify solutions, the numberis given in the "Number of Solutions" column. The "Evaluated Phenotypes" column indicates the results from phenotyping experiments if conducted in this study. The"Min # Rxns" column indicates how many reactions (metabolic and transport) are needed, for cases with multiple solutions this number is the smallestnumber of reactions that are needed.

## **1. Environments with Identifiable Solutions**

BIOIOG	BIOIOG						win. #
Well ID	Result	Source	Compound Name	Kegg ID	Number of Solutions	Evaluated Phenotypes	Rxns
PM1 C12	Strong	Carbon	thymidine	C00214	Only 10 Solutions Identified	Confirmed batch growth	1
PM1 G11	Strong	Carbon	D-malate	C00497	Only 2 Solutions Identified	Confirmed batch growth	2
PM1 C2	Strong	Carbon	D-galactonate-gamma-lactone	C03383	Only 1 Solution Identified	Confirmed batch growth	2
PM1 H9	Strong	Carbon	L-galactonate-gamma-lactone	C01115	>15 Solutions Identified	Confirmed batch growth	6
PM3 B5	Strong	Nitrogen	L-leucine	C00123	>15 Solutions Identified	Not Tested	2
PM3 B8	Strong	Nitrogen	L-phenylalanine	C00079	>15 Solutions Identified	Not Tested	1
PM3 B6	Strong	Nitrogen	L-lysine	C00047	>15 Solutions Identified	Not Tested	2
PM3 B7	Strong	Nitrogen	L-methionine	C00073	>15 Solutions Identified	Not Tested	2
PM3 C1	Strong	Nitrogen	L-tyrosine	C00082	>15 Solutions Identified	Not Tested	1
PM3 G1	Strong	Nitrogen	xanthine	C00385	>15 Solutions Identified	No batch growth observed	1
PM3 G2	Strong	Nitrogen	xanthosine	C01762	>15 Solutions Identified	No batch growth observed	1
PM3 G9	Strong	Nitrogen	e-amino-N-caproate	C02378	>15 Solutions Identified	Not Tested	7
PM3 G11	Strong	Nitrogen	D-amino-N-valerate	C00431	>15 Solutions Identified	Not Tested	3
PM3 C10	Strong	Nitrogen	L-citrulline	C00327	Only needs transporter (1 solution)	Confirmed batch growth	1
PM1 F10	Strong	Carbon	glyoxylate	C00048	Only needs transporter (1 solution)	Confirmed batch growth	1
PM2 E12	Strong	Carbon	5-keto-D-gluconate	C01062	Only needs transporter (1 solution)	Confirmed batch growth	1
PM1 F7	Mutants	Carbon	Propionate	C00163	Only needs transporter (1 solution)	Confirmed batch growth	1
PM1 E7	Weak	Carbon	alpha-hydroxy butyrate	C05984	Only 1 Solution Identified	No batch growth observed	2
PM1 H6	Weak	Carbon	L-lyxose	C01508	Only 12 Solutions Identified	No batch growth observed	3
PM1 D7	Weak	Carbon	alpha-ketobutyrate	C00109	Only needs transporter (1 solution)	No batch growth observed	1
PM2 F5	Weak	Carbon	oxalomalate	C01990	Only 1 Solution Identified	Not Tested	2
PM3 D12	Weak	Nitrogen	agmatine	C00179	Only needs transporter (1 solution)	Confirmed batch growth	1
PM3 B3	Weak	Nitrogen	L-histidine	C00135	>15 Solutions Identified	Not Tested	2
PM3 B4	Weak	Nitrogen	L-isoleucine	C00407	>15 Solutions Identified	Not Tested	1

#### 1. (cont.) Environments with Identifiable Solutions Biolog Biolog

Biolog	Biolog						Min. #
Well ID	Result	Source	Compound Name	Kegg ID	Number of Solutions	Evaluated Phenotypes	Rxns
PM2 A8	Weak	Carbon	glycogen	C00182	Only needs transporter (1 solution)	Not Tested	1
PM2 E10	Weak	Carbon	beta-hydroxy pyruvat	e C00168	Only needs transporter (1 solution)	Not Tested	1

## 2. Environments with No Solutions or Not in Universal Database (U) Biolog Biolog

Well ID	Result	Source	Compound Name	Kegg ID	Number of Solutions
PM1 D10	Strong	Carbon	lactulose	C07064	Metabolite not in U
PM3 E10	Strong	Nitrogen	D-mannosamine	C03570	Metabolite not in U
PM2 C7	Strong	Carbon	b-methyl-D-galactoside	C03619	No Solutions
PM2 A6	Strong	Carbon	Dextrin	C00721	Metabolite not in U
PM1 H5	Weak	Carbon	Psicose	C06468	Metabolite not in U
PM2 A12	Weak	Carbon	pectin	C00714	Metabolite not in U
PM3 C4	Weak	Nitrogen	D-asparagine	C01905	Metabolite not in U

# 3. Environments with No Matching Kegg ID Biolog Biolog

•	•			
Well ID	Result	Source	Compound Name	
PM1 G6	Strong	Carbon	L-alanyl-glycine	Metabolite not in U
PM1 G9	Strong	Carbon	Mono Methyl Succinate	Metabolite not in U
PM1 G10	Strong	Carbon	methyl pyruvate	Metabolite not in U
PM1 D8	Strong	Carbon	alpha-methyl-D-galactoside	Metabolite not in U
PM2 F1	Strong	Carbon	D-lactic acid methyl ester	Metabolite not in U
PM3 E6	Strong	Nitrogen	Glucuronamide	Metabolite not in U
PM1 F1	Strong	Carbon	glycyl-L-aspartate	Metabolite not in U
PM1 E8	Weak	Carbon	beta-methyl-D-glucoside	Metabolite not in U
PM1 F6	Weak	Carbon	bromo-succinate	Metabolite not in U
PM2 A4	Weak	Carbon	beta-cyclodextrin	Metabolite not in U
PM2 B12	Weak	Carbon	3-0-b-D-galactopyranosyl-D-arabinose	Metabolite not in U
PM2 C8	Weak	Carbon	3-methyl glucose	Metabolite not in U
PM3 D2	Weak	Nitrogen	N-phthaloyl-L-glutamate	Metabolite not in U
PM3 G4	Weak	Nitrogen	alloxan	Metabolite not in U
PM1 G1	Weak	Carbon	glycyl-L-glutamate	Metabolite not in U
PM1 H1	Weak	Carbon	glycyl-L-proline	Metabolite not in U

### 4. Previous Experiments Contradict Biolog Results

BiologBiologWell IDResultSourceCompound NamePM1 B10WeakCarbonformate

### 5. Environments Which Can Be Resolved By Current Literature

Biolog	Biolog				
Well ID	Result	Source	Compound Name	Kegg ID	Number of Solutions
PM2 B3	Strong	Carbon	beta-D-allose	C01487	Metabolite not in U
PM1 E3	Strong	Carbon	glucose-1-phosphate	C00103	Needs transport mechanism
PM1 E4	Strong	Carbon	fructose-6-phosphate	C00085	Needs transport mechanism
PM3 A11	Strong	Nitrogen	L-cysteine	C00097	>15 Solutions Identified