

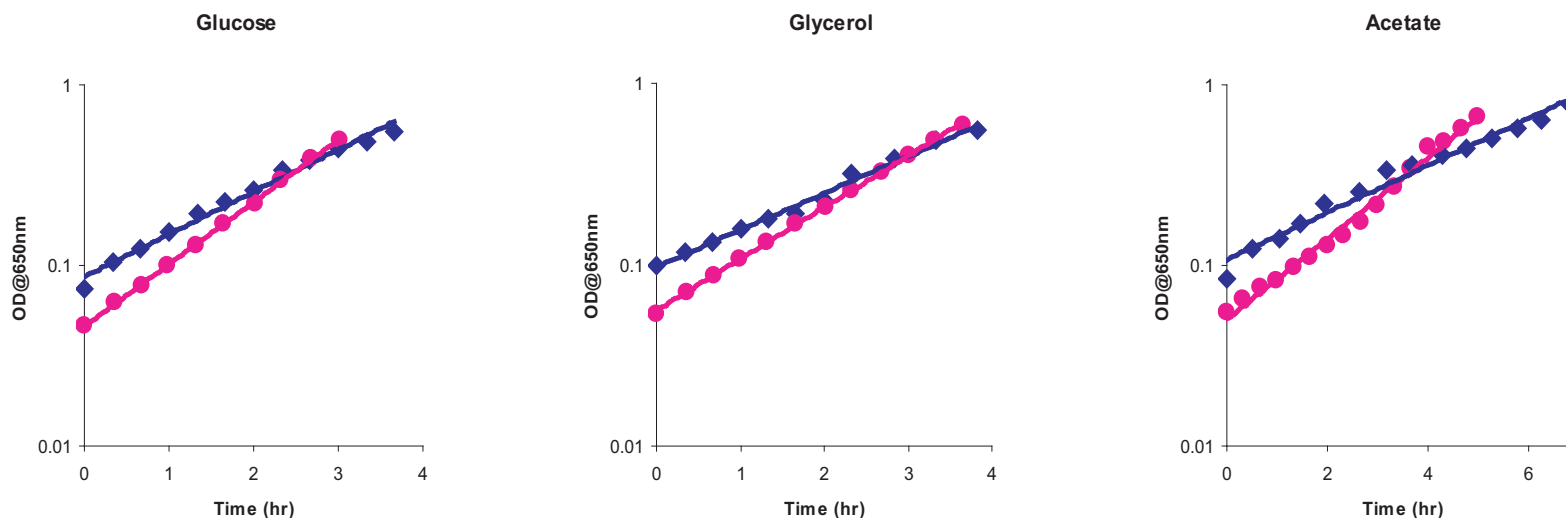
Absolute Metabolite Concentrations and Implied Enzyme Active Site Occupancy in *Escherichia coli*

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Supplementary Figure 1: Growth rates. Comparison of growth rates for the filter cultures (blue) to those in comparable liquid media (pink). (a) glucose (b) glycerol, (c) acetate



Supplementary Table 1: Completeness of labeling of ^{13}C -grown cultures. Completeness of labeling was determined for groups of compounds that label identically. Confidence limits were calculated by propagation of uncertainty in logarithmic space, as described previously.¹

Group	Percentage fully labeled	Upper bound 95% Confidence	Lower bound 95% Confidence
glycolysis	98%	100%	97%
aspartate	80%	81%	79%
purine	45%	48%	42%
pyrimidines	21%	21%	20%
NAD	41%	45%	38%
succinate/fumarate	97%	99%	95%

Supplementary Table 2: Class assignments of metabolites used in determining completeness of labeling

Compound Name	Labeling group
2,3-dihydroxybenzoic acid	glycolysis
3-phosphoglycerate	glycolysis
6-phospho-gluconate	glycolysis
acetoacetyl-CoA	NAD
acetyl-CoA	NAD
acetylphosphate	glycolysis
aconitate	aspartate
adenine	purine
adenosine	purine
adenosine-phosphosulfate	purine
ADP	NAD
ADP-Glucose	purine
alanine	glycolysis
α -ketoglutarate	aspartate
AMP	purine
anthranillate	glycolysis
arginine	pyrimidine
asparagine	aspartate
aspartate	aspartate
ATP	purine
carbamyl-aspartate	pyrimidine
citrate	aspartate
citrulline	pyrimidine
CMP	pyrimidine
coenzyme A	NAD
CTP	pyrimidine
cyclic-AMP	purine
cytidine	pyrimidine
cytosine	pyrimidine
dAMP	purine

dATP	purine
dCTP	pyrimidine
deoxyadenosine	purine
deoxyguanosine	purine
deoxyribose-5-P	glycolysis
gluconate	glycolysis
dGMP	purine
DHAP	glycolysis
dihydroorotate	pyrimidine
FAD	purine ^{2a}
FBP	glycolysis
flavin nucleotide	purine ^{2a}
fumarate	succinate
GDP	purine
gluconolactone	glycolysis
glucosamine-6-phosphate	glycolysis
glutamate	aspartate
glutamine	aspartate
glutathione	aspartate
glutathione disulphide	asp ^{2a}
glycerate	glycolysis
glycerol-3-phosphate	glycolysis
GMP	purine
GTP	purine
guanine	purine
guanosine	purine
hexose-P	glycolysis
histidine	glycolysis
histidinol	glycolysis
homocysteine	aspartate
inosine-diphosphate	purine
inosine-monophosphate	purine
inosine-triphosphate	purine

isoleucine+leucine	avg(asp ,glycolysis) ^b
lysine	aspartate
malate	aspartate
malonyl-CoA	NAD
methionine	aspartate
myo-inositol	glycolysis
N-acetyl-glucosamine-1P	glycolysis
N-acetyl-ornithine	aspartate
NAD ⁺	NAD
NADH	NAD
NADP ⁺	NAD
NADPH	NAD
Ornithine	aspartate
p-hydroxybenzoate	glycolysis
pentose-P	glycolysis
phenylalanine	glycolysis
phenylpyruvate	glycolysis
phosphoenolpyruvate	glycolysis
proline	aspartate
propionyl-CoA	NAD
PRPP	glycolysis
quinolinate	aspartate
riboflavin	purine
S-adenosyl-L-methionine	aspartate
serine	glycolysis
shikimate	glycolysis
succinate	succinate
succinyl-CoA	NAD
TDP	pyrimidine
threonine	aspartate
tryptophan	glycolysis
TTP	pyrimidine
tyrosine	glycolysis

UDP	pyrimidine
UDP- glucose	pyrimidine
UDP-glucaronate	pyrimidine
UDP-N-acetyl-D- glucosamine	pyrimidine
uridine	pyrimidine
UTP	pyrimidine
valine	glycolysis

^aFor metabolites whose carbon atoms originate from multiple groups, the fraction universally labeled was determined as the product of the fractional labeling of each group.

^bAverage for isoleucine and leucine, which originate from different labeling groups but were analytically indistinguishable in the present LC-MS/MS method.

Supplementary Table 3: Absolute intracellular concentrations of metabolites

Best estimates of concentrations of metabolites grown with glucose, glycerol, or acetate as the carbon source, with 95% confidence intervals of the concentrations in parentheses. The column “significance” provides information regarding whether the concentration differences between glucose, glycerol, and acetate-grown cells were statistically significant at a false discovery rate of 0.05; 1 = glucose and glycerol significantly different, 2 = glucose and acetate significantly different, 3 = glycerol and acetate significantly different.

	Glucose (M)	Glycerol (M)	Acetate (M)	Significance ^a
2,3-Dihydroxybenzoic acid	1.38E-4 (1.17E-4 to 1.64E-4)	4.14E-4 (2.91E-4 to 5.88E-4)	2.74E-4 (1.26E-4 to 5.97E-4)	1,2,3
3-phosphoglycerate ^b	1.54E-3 (1.51E-3 to 1.58E-3)	4.08E-3 (3.36E-3 to 4.95E-3)	1.51E-3 (1.02E-3 to 2.23E-3)	1,3
4-hydroxybenzoate	5.22E-5 (4.43E-5 to 6.15E-5)	7.87E-4 (3.67E-4 to 1.69E-3)	2.94E-4 (1.66E-4 to 5.19E-4)	1,2,3
6-phospho-D-gluconate	3.77E-3 (3.69E-3 to 3.85E-3)	4.00E-4 (2.46E-4 to 6.53E-4)	1.93E-4 (9.00E-5 to 4.14E-4)	1
acetoacetyl-CoA	2.18E-5 (1.37E-5 to 3.47E-5)			
acetyl-CoA	6.06E-4 (5.29E-4 to 6.94E-4)	7.34E-4 (3.32E-4 to 1.63E-3)	6.28E-4 (2.27E-4 to 1.74E-3)	1,2
acetylphosphate	1.07E-3 (1.02E-3 to 1.13E-3)	1.05E-3 (3.90E-4 to 2.80E-3)	1.56E-3 (1.20E-3 to 2.04E-3)	2
aconitate	1.61E-5 (1.38E-5 to 1.88E-5)	9.59E-5 (5.27E-5 to 1.74E-4)	7.23E-5 (3.66E-5 to 1.43E-4)	1,2,3
adenine	1.47E-6 (1.26E-6 to 1.71E-6)			
adenosine	1.31E-7 (9.44E-8 to 1.82E-7)			
adenosine-phosphosulfate	6.63E-6 (5.10E-6 to 8.62E-6)			
ADP	5.55E-4 (4.37E-4 to 7.04E-4)	1.49E-4 (9.81E-5 to 2.25E-4)	1.89E-4 (8.02E-5 to 4.47E-4)	
ADP-glucose	4.27E-6 (2.83E-6 to 6.44E-6)			
alanine	2.55E-3 (2.32E-3 to 2.80E-3)	1.77E-3 (1.49E-3 to 2.09E-3)	8.79E-4 (7.10E-4 to 1.09E-3)	1,2,3
α-ketoglutarate	4.43E-4 (3.12E-4 to 6.31E-4)	6.16E-4 (3.57E-4 to 1.06E-3)	4.46E-4 (2.65E-4 to 7.51E-4)	1
AMP	2.81E-4 (2.32E-4 to 3.41E-4)	1.56E-4 (9.41E-5 to 2.60E-4)	1.01E-4 (3.16E-5 to 3.25E-4)	1
anthranilate	3.48E-6 (3.34E-6 to 3.62E-6)	2.66E-6 (1.43E-6 to 4.93E-6)	3.44E-6 (2.63E-6 to 4.49E-6)	
arginine	5.69E-4 (4.79E-4 to 6.75E-4)			
asparagine	5.11E-4 (4.42E-4 to 5.92E-4)	9.70E-4 (6.09E-4 to 1.55E-3)	5.40E-4 (3.32E-4 to 8.80E-4)	1
aspartate	4.23E-3 (3.56E-3 to 5.04E-3)	9.30E-3 (6.45E-3 to 1.34E-2)	7.35E-3 (3.27E-3 to 1.65E-2)	1,2
ATP	9.63E-3 (8.13E-3 to 1.14E-2)	8.98E-3 (6.91E-3 to 1.17E-2)	4.14E-3 (2.79E-3 to 6.13E-3)	1,2,3
carbamyl-aspartate	5.90E-4 (3.64E-4 to 9.55E-4)	9.02E-5 (3.20E-5 to 2.54E-4)	2.66E-5 (6.33E-6 to 1.12E-4)	
citrate	1.96E-3 (1.10E-3 to 3.48E-3)	2.32E-3 (1.23E-3 to 4.37E-3)	2.19E-2 (1.75E-2 to 2.73E-2)	1,2,3

	Glucose (M)	Glycerol (M)	Acetate (M)	Significance ^a
citrulline	1.35E-3 (1.23E-3 to 1.48E-3)	9.31E-4 (6.24E-4 to 1.39E-3)	5.16E-4 (3.85E-4 to 6.90E-4)	1,2,3
CMP	3.60E-4 (1.87E-4 to 6.94E-4)			
coenzyme-A	1.37E-3 (8.83E-5 to 2.12E-2)	4.54E-3 (2.65E-4 to 7.78E-2)	3.70E-3 (2.17E-4 to 6.30E-2)	1,2,3
CTP	2.73E-3 (2.27E-3 to 3.27E-3)	1.40E-3 (5.64E-4 to 3.49E-3)	1.15E-3 (4.75E-4 to 2.77E-3)	1,2
cyclic-AMP	3.52E-5 (2.82E-5 to 4.39E-5)	8.30E-5 (3.04E-5 to 2.26E-4)	1.46E-4 (5.37E-5 to 3.98E-4)	1,2,3
cytidine	2.59E-6 (1.18E-6 to 5.67E-6)			
cytosine	1.41E-5 (8.65E-6 to 2.29E-5)			
dAMP	8.84E-6 (2.99E-6 to 2.62E-5)			
dATP	1.55E-5 (8.13E-6 to 2.96E-5)	5.10E-5 (2.50E-5 to 1.04E-4)	6.85E-5 (1.90E-5 to 2.46E-4)	1,2,3
dCTP	3.45E-5 (2.60E-5 to 4.57E-5)			
deoxyadenosine	2.82E-6 (1.80E-6 to 4.41E-6)			
deoxyguanosine	5.22E-7 (4.11E-7 to 6.62E-7)			
deoxyribose-5-P	3.03E-4 (4.82E-5 to 1.90E-3)			
dGMP	5.07E-5 (3.91E-5 to 6.58E-5)			
dihydroorotate	1.19E-5 (1.16E-5 to 1.23E-5)	4.58E-6 (3.34E-6 to 6.28E-6)	3.59E-6 (2.44E-6 to 5.28E-6)	1,2,3
dihydroxyacetone-phosphate	3.74E-4 (3.44E-4 to 4.05E-4)	5.44E-4 (3.72E-4 to 7.95E-4)	1.47E-4 (6.74E-5 to 3.19E-4)	1
dTDP	3.78E-4 (3.37E-4 to 4.26E-4)			
dTTP	4.62E-3 (4.21E-3 to 5.08E-3)	5.25E-4 (3.84E-4 to 7.17E-4)	6.67E-4 (5.56E-4 to 8.01E-4)	2
FAD	1.73E-4 (9.33E-5 to 3.19E-4)	2.09E-4 (4.23E-5 to 1.04E-3)	4.93E-5 (6.56E-6 to 3.71E-4)	1,3
flavin mononucleotide	5.37E-5 (3.84E-5 to 7.51E-5)	2.74E-6 (5.30E-7 to 1.42E-5)	5.31E-5 (1.27E-5 to 2.23E-4)	2,3
fructose-1,6-bisphosphate	1.52E-2 (1.40E-2 to 1.64E-2)	5.85E-3 (4.19E-3 to 8.16E-3)	<1.52E-4	1,2,3
fumarate	1.15E-4 (3.00E-6 to 4.42E-3)	2.55E-4 (6.58E-6 to 9.92E-3)	2.67E-4 (6.73E-6 to 1.06E-2)	1,2
GDP	6.76E-4 (4.99E-4 to 9.16E-4)	2.32E-5 (5.73E-6 to 9.40E-5)	1.77E-5 (4.64E-6 to 6.74E-5)	
gluconate	4.16E-5 (5.74E-6 to 3.02E-4)			
gluconolactone	1.04E-3 (6.47E-4 to 1.68E-3)			
glucosamine-6_phosphate	1.15E-3 (9.59E-4 to 1.39E-3)	3.78E-4 (9.94E-5 to 1.44E-3)	3.16E-4 (2.18E-4 to 4.58E-4)	
glutamate	9.60E-2 (9.24E-2 to 9.98E-2)	1.49E-1 (1.33E-1 to 1.68E-1)	4.48E-2 (3.26E-2 to 6.14E-2)	1,3
glutamine	3.81E-3 (3.50E-3 to 4.15E-3)	4.95E-3 (4.35E-3 to 5.63E-3)	3.06E-3 (2.62E-3 to 3.57E-3)	1,2,3
glutathione	1.66E-2 (1.53E-2 to 1.79E-2)	1.76E-2 (1.51E-2 to 2.06E-2)	7.97E-3 (5.49E-3 to 1.16E-2)	1,3
glutathione disulfide	2.37E-3 (1.94E-3 to 2.90E-3)	7.31E-3 (2.87E-3 to 1.86E-2)	1.68E-3 (8.67E-4 to 3.26E-3)	1,3

	Glucose (M)	Glycerol (M)	Acetate (M)	Significance ^a
glycerate	1.41E-3 (6.44E-4 to 3.08E-3)			
glycerolphosphate	4.90E-5 (1.29E-5 to 1.87E-4)	8.13E-4 (1.82E-4 to 3.63E-3)	2.46E-4 (4.94E-5 to 1.23E-3)	1,2,3
GMP	2.37E-5 (1.66E-5 to 3.38E-5)			
GTP	4.87E-3 (1.57E-3 to 1.51E-2)	2.69E-3 (7.03E-4 to 1.03E-2)	1.25E-3 (3.64E-4 to 4.28E-3)	
guanine	1.88E-4 (1.32E-4 to 2.69E-4)	3.31E-6 (1.22E-6 to 9.00E-6)	< 3.31E-6	2,3
guanosine	1.62E-6 (1.22E-6 to 2.17E-6)			
hexose-P ^c	8.75E-3 (8.43E-3 to 9.08E-3)	5.48E-3 (4.43E-3 to 6.79E-3)	4.36E-3 (3.51E-3 to 5.42E-3)	1,2
histidine	6.76E-5 (4.58E-5 to 9.97E-5)	1.75E-4 (9.78E-5 to 3.13E-4)	9.75E-5 (4.39E-5 to 2.17E-4)	1,3
histidinol	1.28E-5 (1.26E-5 to 1.30E-5)	1.92E-5 (2.99E-6 to 1.23E-4)	1.96E-5 (8.90E-6 to 4.31E-5)	
homocysteine	3.70E-4 (3.65E-4 to 3.75E-4)			
inosine-diphosphate	2.38E-5 (1.76E-5 to 3.22E-5)			
inosine-monophosphate	2.72E-4 (1.73E-4 to 4.26E-4)			
inosine-triphosphate	2.05E-4 (1.38E-4 to 3.03E-4)			
isoleucine+leucine	3.03E-4 (2.98E-4 to 3.08E-4)	4.38E-4 (3.50E-4 to 5.48E-4)	3.41E-4 (2.82E-4 to 4.13E-4)	1,2,3
lysine	4.05E-4 (3.27E-4 to 5.02E-4)	7.62E-4 (5.51E-4 to 1.06E-3)	5.54E-4 (4.14E-4 to 7.43E-4)	1,2,3
malate	1.68E-3 (1.66E-3 to 1.70E-3)	3.45E-3 (3.07E-3 to 3.89E-3)	3.33E-3 (2.95E-3 to 3.76E-3)	1,2,3
malonyl-CoA	3.54E-5 (4.05E-7 to 3.09E-3)			
methionine	1.45E-4 (1.31E-4 to 1.61E-4)	1.29E-4 (9.87E-5 to 1.68E-4)	6.59E-5 (4.38E-5 to 9.92E-5)	1
myo-inositol	5.72E-6 (4.22E-6 to 7.75E-6)	7.05E-6 (4.26E-6 to 1.17E-5)	6.90E-6 (3.92E-6 to 1.22E-5)	
N-acetyl-glucosamine-1P	8.19E-5 (7.25E-5 to 9.26E-5)			
N-acetyl-ornithine	4.33E-5 (2.71E-5 to 6.94E-5)	3.98E-4 (9.18E-5 to 1.72E-3)	1.52E-4 (4.31E-5 to 5.35E-4)	1,2,3
NAD	2.55E-3 (2.32E-3 to 2.80E-3)	4.08E-3 (1.28E-3 to 1.30E-2)	2.43E-3 (1.11E-3 to 5.33E-3)	1,2
NADH	8.32E-5 (5.45E-5 to 1.27E-4)	1.29E-4 (1.94E-5 to 8.55E-4)	1.35E-4 (7.91E-5 to 2.31E-4)	1,2
NADP+	2.08E-6 (1.40E-7 to 3.11E-5)			
NADPH	1.21E-4 (1.10E-4 to 1.34E-4)	2.88E-4 (4.04E-5 to 2.05E-3)	2.98E-4 (5.22E-5 to 1.70E-3)	1
ornithine	1.01E-5 (6.81E-6 to 1.51E-5)	3.03E-5 (8.47E-6 to 1.08E-4)	6.40E-5 (3.42E-5 to 1.20E-4)	1,2,3
pentose-P ^d	1.32E-3 (9.83E-4 to 1.77E-3)	1.02E-3 (6.83E-4 to 1.52E-3)	6.86E-4 (4.44E-4 to 1.06E-3)	2
phenylalanine	1.82E-5 (1.77E-5 to 1.87E-5)	4.21E-5 (3.38E-5 to 5.25E-5)	2.74E-5 (2.03E-5 to 3.70E-5)	1,2,3
phenylpyruvate	8.98E-5 (5.01E-5 to 1.61E-4)	1.95E-4 (1.00E-4 to 3.79E-4)	1.48E-4 (1.54E-5 to 1.42E-3)	1,2,3
phosphoenolpyruvate	1.84E-4 (1.46E-4 to 2.31E-4)	1.34E-3 (1.04E-3 to 1.73E-3)	9.09E-4 (6.59E-4 to 1.25E-3)	1,2,3

	Glucose (M)	Glycerol (M)	Acetate (M)	Significance ^a
proline	3.85E-4 (3.72E-4 to 3.99E-4)	4.51E-4 (4.03E-4 to 5.05E-4)	3.61E-4 (3.10E-4 to 4.21E-4)	1,2,3
propionyl-CoA	5.32E-6 (3.88E-6 to 7.29E-6)			
PRPP	2.58E-4 (1.36E-4 to 4.92E-4)	1.53E-4 (7.88E-5 to 2.97E-4)	9.44E-5 (4.84E-5 to 1.84E-4)	1,2
quinolinate	1.15E-5 (2.41E-6 to 5.49E-5)	4.66E-6 (9.11E-7 to 2.38E-5)	8.96E-7 (1.52E-7 to 5.28E-6)	
riboflavin	1.90E-5 (1.72E-5 to 2.11E-5)	2.21E-5 (1.40E-5 to 3.48E-5)	1.88E-5 (1.35E-5 to 2.61E-5)	1,2
S-adenosyl-L-methionine	1.84E-4 (1.19E-4 to 2.84E-4)	1.10E-3 (3.85E-4 to 3.16E-3)	3.88E-4 (1.28E-4 to 1.18E-3)	1,2,3
serine	6.80E-5 (2.57E-5 to 1.80E-4)	1.50E-4 (5.26E-5 to 4.27E-4)	9.55E-5 (3.54E-5 to 2.58E-4)	1,2,3
shikimate	1.41E-5 (7.02E-6 to 2.81E-5)			
succinate	5.69E-4 (3.41E-4 to 9.49E-4)	1.14E-3 (6.26E-4 to 2.09E-3)	9.23E-4 (3.90E-4 to 2.19E-3)	1
succinyl-CoA	2.33E-4 (1.42E-4 to 3.83E-4)	1.44E-3 (3.15E-4 to 6.61E-3)	3.90E-4 (9.88E-5 to 1.54E-3)	1,2,3
threonine	1.79E-4 (1.74E-4 to 1.85E-4)			
tryptophan	1.21E-5 (1.14E-5 to 1.29E-5)	2.36E-5 (1.87E-5 to 2.98E-5)	2.05E-5 (1.26E-5 to 3.33E-5)	1,2
tyrosine	2.89E-5 (1.87E-5 to 4.47E-5)	8.74E-5 (4.89E-5 to 1.56E-4)	5.22E-5 (2.82E-5 to 9.64E-5)	1,2,3
UDP	1.79E-3 (1.18E-3 to 2.72E-3)			
UDP-glucuronate	5.66E-4 (1.20E-4 to 2.67E-3)	3.75E-4 (7.88E-5 to 1.79E-3)	1.88E-4 (3.93E-5 to 8.98E-4)	1,2,3
UDP-glucose	2.50E-3 (1.23E-3 to 5.11E-3)	1.12E-3 (5.39E-4 to 2.32E-3)	5.53E-4 (2.63E-4 to 1.16E-3)	1,2,3
UDP-N-acetyl-glucosamine	9.24E-3 (6.79E-3 to 1.26E-2)	4.11E-3 (2.93E-3 to 5.77E-3)	2.40E-3 (1.67E-3 to 3.43E-3)	1,2,3
uridine	2.09E-3 (1.96E-3 to 2.24E-3)			
UTP	8.29E-3 (7.76E-3 to 8.86E-3)	3.99E-3 (3.49E-3 to 4.56E-3)	2.37E-3 (1.85E-3 to 3.05E-3)	1,2,3
valine	4.02E-3 (3.53E-3 to 4.58E-3)	2.29E-3 (1.70E-3 to 3.08E-3)	1.07E-3 (8.17E-4 to 1.41E-3)	1,2

^a1 denotes a significant difference in concentration between glucose and glycerol cultures, 2 between glucose and acetate cultures, and 3 between glycerol and acetate cultures.

^b3-phosphoglycerate concentration may be overestimated due to degradation of 1,3-diphosphoglycerate to 3-phosphoglycerate during sample handling.

^chexose-P denotes the combined pools of fructose-6-phosphate, glucose-6-phosphate and glucose-1-phosphate (**112**)

^dpentose-P denotes the combined pools of ribose-5-phosphate, ribulose-5-phosphate, and xyulose-5-phosphate

Supplementary Table 4: Fraction of metabolite signal found to be extracellular

The molar fraction of metabolites found to be extracellular. Extracellular volume was ~300-fold intracellular volume. For compounds not listed in this table (or where the table entry is blank), the extracellular fraction was < 10%.

	glucose	glycerol	acetate
glycerol-phosphate		45%	
2,3-dihydroxybenzoate		28%	26%
p-hydroxybenzoate	82%	59%	49%
phenylpyruvate	33%	40%	19%
cAMP		79%	40%
citrate	10%	18%	21%
aconitate			44%

Supplementary Table 5: Literature values of K' from which ΔG^0 were derived

Reactant	Product	K'	Reference
GAP	DHAP	24	Meyerhof, O.; Junowicz-Kocholaty, R.; J. Biol. Chem.; 149, 71 (1943).
GAP	DHAP	25	Meyerhof, O.; Junowicz-Kocholaty, R.; J. Biol. Chem.; 149, 71 (1943).
GAP	DHAP	21	Meyerhof, O.; Junowicz-Kocholaty, R.; J. Biol. Chem.; 149, 71 (1943).
GAP	DHAP	17	Meyerhof, O.; Oesper, P.; J. Biol. Chem.; 170, 1 (1947).
GAP	DHAP	22	Burton, P.M.; Waley, S.G.; Biochim. Biophys. Acta; 151, 714 (1968).
GAP	DHAP	22	Lim, W.A.; Raines, R.T.; Knowles, J.R.; Biochemistry; 27, 1158 (1988).
GAP	DHAP	22	Oesper, P.; Meyerhof, O.; Arch. Biochem. Biophys.; 27, 223 (1950).
			Veech, R.L.; Rajjman, L.; Dalziel, K.; Krebs, H.A.; Biochem. J.; 115, 837 (1969).
GAP	DHAP	22	
GAP	DHAP	19	Krietsch, W.K.G.; Methods Enzymol.; 41, 434 (1975).
FBP	DHAP	.0064	Utter, M.F.; Werkman, C.H.; J. Bacteriol.; 42, 665 (1941).
FBP	DHAP	0.003	Meyerhof, O.; Junowicz-Kocholaty, R.; J. Biol. Chem.; 149, 71 (1943).
FBP	DHAP	0.013	Meyerhof, O.; Lohmann, K.; Biochem. Z.; 271, 89 (1934).
FBP	DHAP	0.0015	Meyerhof, O.; Lohmann, K.; Biochem. Z.; 271, 89 (1934).
FBP	DHAP	0.019	Meyerhof, O.; Biochem. Z.; 277, 77 (1935).
FBP	DHAP	0.00014	Meyerhof, O.; Biochem. Z.; 277, 77 (1935).
			Lehninger, A.L.; Sice, J.; Jensen, E.V.; Biochim. Biophys. Acta; 17, 286 (1955).
FBP	DHAP + GAP	1.18E-04	Herbert, D.; Gordon, H.; Subrahmanyam, V.; Green, D.E.; Biochem. J.; 34, 1108 (1940).
FBP	DHAP + GAP	1.19E-04	Kurski, M.D.; Kondratyook, T.P.; Litvinenko, L.T.; Kosterin, S.O.; Dopov. Akad. Nauk. Ukr. RSR, Ser. B: Geol. Geofiz. Khim. Biol.; 3, 256 (1975).
FBP	DHAP + GAP	5.20E-04	Meyerhof, O.; Junowicz-Kocholaty, R.; J. Biol. Chem.; 149, 71 (1943).
FBP	DHAP + GAP	1.10E-04	Lowry, O.H.; Passonneau, J.V.; J. Biol. Chem.; 239, 31 (1964).
			Veech, R.L.; Rajjman, L.; Dalziel, K.; Krebs, H.A.; Biochem. J.; 115, 837 (1969).
FBP	DHAP + GAP	9.30E-05	
FBP	DHAP + GAP	9.90E-05	Veech, R.L.; Rajjman, L.; Dalziel, K.; Krebs, H.A.; Biochem. J.; 115, 837 (1969).
FBP	DHAP + GAP	5.60E-05	Lehninger, A.L.; Sice, J.; Jensen, E.V.; Biochim. Biophys. Acta; 17, 286 (1955).
FBP	DHAP + GAP	2.80E-06	

FBP	DHAP + GAP	2.80E-06	Kurski, M.D.; Kondratyook, T.P.; Litvinenko, L.T.; Kosterin, S.O.; Dopov. Akad. Nauk. Ukr. RSR, Ser. B: Geol. Geofiz. Khim. Biol.;3, 256 (1975).
FBP + H2O	Pi + F6P	227	Lawson, J.W.R.; Veech, R.L.; J. Biol. Chem.; 254, 6528 (1979).
FBP + H2O	Pi + F6P	174	Lawson, J.W.R.; Veech, R.L.; J. Biol. Chem.; 254, 6528 (1979).
FBP + H2O	Pi + F6P	47	Liu, F.; Fromm, H.J.; J. Biol. Chem.; 266, 11774 (1991).
ATP + F6P	FBP + ADP	2.70E+03	Bvhme, H.-J.; Schellenberger, W.; Hofmann, E.; Acta Biol. Med. Germ.; 34, 15 (1975).
ATP + F6P	FBP + ADP	4.80E+03	Bvhme, H.-J.; Schellenberger, W.; Hofmann, E.; Acta Biol. Med. Germ.; 34, 15 (1975).
ATP + F6P	FBP + ADP	2900	Rao, G.S.J.; Harris, B.G.; Cook, P.F.; J. Biol. Chem.; 262, 14074 (1987).
ATP + F6P	FBP + ADP	232.22774	Streuer
ATP + F6P	FBP + ADP	734.09981	Voet & Voet
ATP + F6P	FBP + ADP	2.29E+03	Hanson, R.L.; Rudolph, F.B.; Lardy, H.A.; J. Biol. Chem.; 248, 7852 (1973).
3PG + ATP	13DPG + ADP	3.18E-04	Cornell, N.W.; Leadbetter, M.; Veech, R.L.; J. Biol. Chem.; 254, 6522 (1979).
3PG + ATP	13DPG + ADP	2.25E-04	Cornell, N.W.; Leadbetter, M.; Veech, R.L.; J. Biol. Chem.; 254, 6522 (1979).
3PG + ATP	13DPG + ADP	1.50E-04	Schmidt, P.P.; Travers, F.; Barman, T.; Biochemistry; 34, 824 (1995).
3PG + ATP	13DPG + ADP	3.10E-04	Blcher, T.; Biochim. Biophys. Acta; 1, 292 (1947). Reference : Blcher, T.; Biochim. Biophys. Acta; 1, 292 (1947)
3PG + ATP	13DPG + ADP	3.00E-04	Krietsch, W.K.G.; Blcher, T.; Eur. J. Biochem.; 17, 568 (1970).
3PG + ATP	13DPG + ADP	2.30E-03	Jaffe, E.K.; Cohn, M.; J. Biol. Chem.; 255, 3240 (1980).
GAP + ADP + NAD + Pi	3PG + NADH + ATP	2.17E+03	Cornell, N.W.; Leadbetter, M.; Veech, R.L.; J. Biol. Chem.; 254, 6522 (1979).
GAP + ADP + NAD + Pi	3PG + NADH + ATP	7.10E+01	Veech, R.L.; "Measurement of Respiratory Metabolism in Animal Tissues"; Thesis, Oxford University (1968).
GAP + ADP + NAD + Pi	3PG + NADH + ATP	2.80E+01	Veech, R.L.; "Measurement of Respiratory Metabolism in Animal Tissues"; Thesis, Oxford University (1968).
GAP + ADP + NAD + Pi	3PG + NADH + ATP	1.93E+03	van der Meer, R.; Akerboom, T.P.M.; Groen, A.K.; Tager, J.M.; Eur. J. Biochem.; 84, 421 (1978).
GAP + ADP + NAD + Pi	3PG + NADH + ATP	3.06E+02	van der Meer, R.; Akerboom, T.P.M.; Groen, A.K.; Tager, J.M.; Eur. J. Biochem.; 84, 421 (1978).
GAP + ADP + NAD + Pi	3PG + NADH + ATP	1.21E+02	Streuer

GAP + ADP + NAD + Pi	3PG + NADH + ATP	6.06E+02	Voet & Voet
2PG	3PG	8.00E+00	Ito, N.; Grisolia, S.; J. Biol. Chem.; 234, 242 (1959).
2PG	3PG	11.3	Hill, B.; Attwood, M.M.; J. Gen. Microbiol.; 96, 185 (1976).
2PG	3PG	9.8	Lowry, O.H.; Passonneau, J.V.; J. Biol. Chem.; 239, 31 (1964).
2PG	3PG	4	Meyerhof, O.; Kiessling, W.; Biochem. Z.; 280, 99 (1935).
2PG	3PG	3.45	Meyerhof, O.; Kiessling, W.; Biochem. Z.; 276, 239 (1935).
2PG	3PG	7.9	Meyerhof, O.; Schulz, W.; Biochem. Z.; 297, 60 (1938).
2PG	3PG	6	Meyerhof, O.; Oesper, P.; J. Biol. Chem.; 179, 1371 (1949).
2PG	3PG	4.9	Chiba, H.; Sugimoto, E.; Bull. Agric. Chem. Soc. Jpn.; 23, 207 (1959).
2PG	3PG	5.35	Chiba, H.; Sugimoto, E.; Bull. Agric. Chem. Soc. Jpn.; 23, 207 (1959).
2PG	3PG	5	Cowgill, R.W.; Pizer, L.I.; J. Biol. Chem.; 223, 885 (1956).
2PG	3PG	5	Grisolia, S.; Carreras, J.; Methods Enzymol.; 42, 435 (1975).
2PG	3PG	5.8	Rodwell, V.W.; Towne, J.C.; Grisolia, S.; J. Biol. Chem.; 228, 875 (1957).
2PG	3PG	6.8	Rodwell, V.W.; Towne, J.C.; Grisolia, S.; J. Biol. Chem.; 228, 875 (1957).
2PG	3PG	1.13E+01	Guynn, R.W.; Arch. Biochem. Biophys.; 218, 14 (1982).
2PG	3PG	1.04E+01	Guynn, R.W.; Arch. Biochem. Biophys.; 218, 14 (1982).
2PG	3PG	8.65	Clarke, J.B.; Birch, M.; Britton, H.G.; Biochem. J.; 139, 491 (1974).
2PG	3PG	11.65	Clarke, J.B.; Birch, M.; Britton, H.G.; Biochem. J.; 139, 491 (1974).
2PG	3PG	6.1	Grisolia, S.; Methods Enzymol.; 5, 236 (1962).
2PG	PEP + H2O	2.3	Akano, R.; Biochem. Z.; 280, 110 (1935).
2PG	PEP + H2O	0.54	Lohman, K.; Meyerhof, O.; Biochem. Z.; 273, 60 (1934).
2PG	PEP + H2O	2.9	Meyerhof, O.; Oesper, P.; J. Biol. Chem.; 179, 1371 (1949).
2PG	PEP + H2O	4.6	Lowry, O.H.; Passonneau, J.V.; J. Biol. Chem.; 239, 31 (1964).
2PG	PEP + H2O	1.43	Warburg, O.; Christian, W.; Biochem. Z.; 310, 384 (1941).
2PG	PEP + H2O	5.15E+00	Wold, F.; Ballou, C.E.; J. Biol. Chem.; 227, 301 (1957).
2PG	PEP + H2O	2.15E+00	Wold, F.; Ballou, C.E.; J. Biol. Chem.; 227, 301 (1957).
Pyruvate + ATP	PEP +ADP	1.55E-04	Reference : McQuate, J.T.; Utter, M.F.; J. Biol. Chem.; 234, 2151 (1959).
Pyruvate + ATP	PEP +ADP	1.10E-03	Reference : McQuate, J.T.; Utter, M.F.; J. Biol. Chem.; 234, 2151 (1959).
Pyruvate + ATP	PEP +ADP	3.00E-04	Reference : Rao, B.D.N.; Kayne, F.J.; Cohn, M.; J. Biol. Chem.; 254, 2689 (1979).

Supplementary Table 6: Thermodynamics of glycolysis on three different carbon sources

Estimates of ΔG^0_{ec} and the free energy of reactions, determined by assuming the flux direction. Calculations assumed a concentration of 20 mM inorganic phosphate², and distribution of hexose-P between glucose-6-phosphate and fructose-6-phosphate, and phosphoglycerate between 2-phosphoglycerate and 3-phosphoglycerate, according to equilibrium.

From	To	ΔG^0_{ec} (kJ/mol)	ΔG glucose (kJ/mol)	ΔG glycerol (kJ/mol)	ΔG acetate (kJ/mol)
ATP + F6P	FBP + ADP	-18.0	-21.1 ± 2.1	-25.7 ± 4.1	-31.9 ± 6.4
FBP	Pi + F6P	-12.0	-26.5 ± 0.5	-25.2 ± 2.3	-16.3 ± 0.5
FBP	DHAP ¹	25.0	-4.2 ± 0.9	0.2 ± 4.3	2.9 ± 7.5
DHAP + NAD + ADP + Pi	3PG + NADH + ATP	-29.0	-2.5 ± 3.6	2.9 ± 15.7	0.4 ± 10.7
3PG	PEP	1.5	-3.5 ± 1.6	-0.8 ± 2	0.8 ± 3.1

¹kJ/mole for FBP and kJ/(2 mole) for DHAP

All values are reported as mean ± 2 standard errors (i.e., 95% confidence intervals) with error estimates based on metabolite concentration data only (i.e., assuming no error in ΔG^0)

Supplementary Table 7: K_m values from the BRENDA database

Enzyme Name	EC#	Compound Name	K _m (moles/L)	Concentration (moles/L)
(2,3-dihydroxybenzoyl)adenylate synthase	2.7.7.58	2,3-dihydroxybenzoate	2.70E-06	1.38E-04
acetyl-CoA C-acyltransferase	2.3.1.16	acetoacetyl-coa	3.10E-05	2.18E-05
3-hydroxyacyl-CoA dehydrogenase	1.1.1.35	acetotacetyl-coa	6.60E-05	2.18E-05
acetate kinase	2.7.2.1	acetyl phosphate	8.94E-04	1.07E-03
acetaldehyde dehydrogenase	1.2.1.10	acetyl-coa	9.54E-06	6.06E-04
glucosamine-1-phosphate N-acetyltransferase	2.3.1.157	acetyl-coa	6.00E-04	6.06E-04
glycine C-acetyltransferase	2.3.1.29	acetyl-coa	5.90E-05	6.06E-04
serine O-acetyltransferase	2.3.1.30	acetyl-coa	5.60E-04	6.06E-04
formate C-acetyltransferase	2.3.1.54	acetyl-coa	5.10E-05	6.06E-04
acetyl-CoA acetyltransferase	2.3.1.9	acetyl-coa	4.70E-04	6.06E-04
citrate (Si)-synthase	2.3.3.1	acetyl-coa	2.16E-04	6.06E-04
malate synthase	2.3.3.9	acetyl-coa	9.00E-06	6.06E-04
acetylornithine deacetylase	3.5.1.16	acetylornithine	1.46E-03	4.33E-05
adenine phosphoribosyltransferase	2.4.2.7	adenine	5.10E-06	1.47E-06
adenine deaminase	3.5.4.2	adenine	8.00E-04	1.47E-06
purine-nucleoside phosphorylase	2.4.2.1	adenosine	2.35E-05	5.55E-04
adenosine deaminase	3.5.4.4	adenosine	7.50E-05	5.55E-04
Phosphoenolpyruvate carboxykinase	4.1.1.49	ADP	5.00E-05	5.55E-04
ADP-sugar diphosphatase	3.6.1.21	ADP-glucose	1.67E-04	4.27E-06
8-amino-7-oxononanoate synthase	2.3.1.47	alanine	6.71E-04	2.55E-03
alanine racemase	5.1.1.1	alanine	2.90E-04	2.55E-03
alanine-tRNA ligase	6.1.1.7	alanine	3.40E-04	2.55E-03
UDP-N-acetylmuramate-L-alanine ligase	6.3.2.8	alanine	4.58E-05	2.55E-03
adenylate kinase	2.7.4.3	AMP	3.80E-05	2.81E-04
5'-nucleotidase	3.1.3.5	AMP	1.54E-04	2.81E-04
AMP nucleosidase	3.2.2.4	AMP	1.34E-03	2.81E-04
adenylyl-sulfate kinase	2.7.1.25	adenosine-phosphosulfate	3.54E-07	6.63E-06
arginine decarboxylase	4.1.1.19	arginine	6.50E-04	5.69E-04
arginine-tRNA ligase	6.1.1.19	arginine	4.96E-05	5.69E-04

asparaginase	3.5.1.1	asparagine	1.29E-05	5.11E-04
asparagine-tRNA ligase	6.1.1.22	asparagine	2.33E-05	5.11E-04
L-aspartate oxidase	1.4.3.16	aspartate	1.43E-03	4.23E-03
aspartate carbamoyltransferase	2.1.3.2	aspartate	9.90E-03	4.23E-03
aspartate transaminase	2.6.1.1	aspartate	2.22E-03	4.23E-03
aromatic-amino-acid transaminase	2.6.1.57	aspartate	4.56E-03	4.23E-03
aspartate kinase	2.7.2.4	aspartate	9.71E-04	4.23E-03
aspartate ammonia-lyase	4.3.1.1	aspartate	1.59E-03	4.23E-03
aspartate-tRNA ligase	6.1.1.12	aspartate	5.48E-05	4.23E-03
phosphoribosylaminoimidazolesuccinocarboxamide synthase	6.3.2.6	aspartate	3.60E-05	4.23E-03
adenylosuccinate synthase	6.3.4.4	aspartate	1.70E-04	4.23E-03
asparagine synthase (glutamine-hydrolysing)	6.3.5.4	aspartate	6.70E-04	4.23E-03
nicotinate phosphoribosyltransferase	2.4.2.11	ATP	4.00E-04	9.63E-03
methionine adenosyltransferase	2.5.1.6	ATP	7.78E-05	9.63E-03
diacylglycerol kinase	2.7.1.107	ATP	1.91E-03	9.63E-03
6-phosphofructokinase	2.7.1.11	ATP	1.18E-04	9.63E-03
gluconokinase	2.7.1.12	ATP	7.44E-05	9.63E-03
ribulokinase	2.7.1.16	ATP	5.92E-05	9.63E-03
glucokinase	2.7.1.2	ATP	3.61E-04	9.63E-03
NAD ⁺ kinase	2.7.1.23	ATP	7.58E-04	9.63E-03
adenylyl-sulfate kinase	2.7.1.25	ATP	1.00E-05	9.63E-03
homoserine kinase	2.7.1.39	ATP	4.06E-04	9.63E-03
hydroxymethylpyrimidine kinase	2.7.1.49	ATP	1.43E-04	9.63E-03
N-acetylglucosamine kinase	2.7.1.59	ATP	1.12E-05	9.63E-03
galactokinase	2.7.1.6	ATP	2.37E-03	9.63E-03
shikimate kinase	2.7.1.71	ATP	1.60E-04	9.63E-03
inosine kinase	2.7.1.73	ATP	6.02E-04	9.63E-03
acetate kinase	2.7.2.1	ATP	2.57E-04	9.63E-03
glutamate 5-kinase	2.7.2.11	ATP	4.47E-04	9.63E-03
aspartate kinase	2.7.2.4	ATP	1.60E-03	9.63E-03
acetylglutamate kinase	2.7.2.8	ATP	5.39E-04	9.63E-03
T2-induced deoxynucleotide kinase	2.7.4.12	ATP	1.01E-03	9.63E-03
cytidylate kinase	2.7.4.14	ATP	3.80E-05	9.63E-03

thiamine-phosphate kinase	2.7.4.16	ATP	2.70E-04	9.63E-03
adenylate kinase	2.7.4.3	ATP	4.80E-05	9.63E-03
phosphomethylpyrimidine kinase	2.7.4.7	ATP	1.93E-04	9.63E-03
dTMP kinase	2.7.4.9	ATP	2.19E-04	9.63E-03
ribose-phosphate diphosphokinase	2.7.6.1	ATP	2.30E-05	9.63E-03
2-amino-4-hydroxy-6-hydroxymethyldihydropteridine diphosphokinase	2.7.6.3	ATP	1.56E-05	9.63E-03
nicotinamide-nucleotide adenyltransferase	2.7.7.1	ATP	5.00E-04	9.63E-03
nicotinate-nucleotide adenyltransferase	2.7.7.18	ATP	5.00E-04	9.63E-03
glucose-1-phosphate adenyltransferase	2.7.7.27	ATP	7.68E-04	9.63E-03
(2,3-dihydroxybenzoyl)adenylate synthase	2.7.7.58	ATP	1.12E-03	9.63E-03
pyruvate, water dikinase	2.7.9.2	ATP	2.80E-05	9.63E-03
phosphoenolpyruvate carboxykinase	4.1.1.49	ATP	6.00E-05	9.63E-03
adenylate cyclase	4.6.1.1	ATP	4.50E-04	9.63E-03
tyrosine-tRNA ligase	6.1.1.1	ATP	5.00E-05	9.63E-03
methionine-tRNA ligase	6.1.1.10	ATP	1.21E-03	9.63E-03
serine-tRNA ligase	6.1.1.11	ATP	6.80E-08	9.63E-03
aspartate-tRNA ligase	6.1.1.12	ATP	6.71E-05	9.63E-03
glycine-tRNA ligase	6.1.1.14	ATP	2.87E-05	9.63E-03
proline-tRNA ligase	6.1.1.15	ATP	6.00E-04	9.63E-03
cysteine-tRNA ligase	6.1.1.16	ATP	2.91E-04	9.63E-03
glutamate-tRNA ligase	6.1.1.17	ATP	1.64E-04	9.63E-03
glutamine-tRNA ligase	6.1.1.18	ATP	7.88E-05	9.63E-03
arginine-tRNA ligase	6.1.1.19	ATP	1.18E-03	9.63E-03
tryptophan-tRNA ligase	6.1.1.2	ATP	2.00E-04	9.63E-03
phenylalanine-tRNA	6.1.1.20	ATP	9.00E-06	9.63E-03
histidine-tRNA ligase	6.1.1.21	ATP	7.06E-04	9.63E-03
asparagine-tRNA ligase	6.1.1.22	ATP	7.60E-05	9.63E-03
threonine-tRNA ligase	6.1.1.3	ATP	2.67E-05	9.63E-03
leucine-tRNA ligase	6.1.1.4	ATP	2.64E-04	9.63E-03
isoleucine-tRNA ligase	6.1.1.5	ATP	4.73E-06	9.63E-03
lysine-tRNA ligase	6.1.1.6	ATP	5.27E-05	9.63E-03
alanine-tRNA ligase	6.1.1.7	ATP	8.30E-05	9.63E-03
long-chain-fatty-acid-[acyl-carrier-protein] ligase	6.2.1.20	ATP	3.00E-04	9.63E-03

O-succinylbenzoate-CoA ligase	6.2.1.26	ATP	7.35E-05	9.63E-03
long-chain-fatty-acid-CoA ligase	6.2.1.3	ATP	3.42E-05	9.63E-03
aspartate-ammonia ligase	6.3.1.1	ATP	2.70E-04	9.63E-03
glutamate-ammonia ligase	6.3.1.2	ATP	2.00E-04	9.63E-03
pantoate-beta-alanine ligase	6.3.2.1	ATP	9.54E-05	9.63E-03
dihydrofolate synthase	6.3.2.12	ATP	6.90E-06	9.63E-03
UDP-N-acetylmuramoyl-L-alanyl-D-glutamate-2,6-diaminopimelate ligase	6.3.2.13	ATP	6.20E-04	9.63E-03
tetrahydrofolate synthase	6.3.2.17	ATP	6.82E-05	9.63E-03
glutamate-cysteine ligase	6.3.2.2	ATP	3.16E-05	9.63E-03
glutathione synthase	6.3.2.3	ATP	9.28E-04	9.63E-03
UDP-N-acetylmuramate-L-alanine ligase	6.3.2.8	ATP	1.39E-04	9.63E-03
phosphoribosylformylglycinamide cyclo-ligase	6.3.3.1	ATP	7.30E-05	9.63E-03
dethiobiotin synthase	6.3.3.3	ATP	5.00E-06	9.63E-03
GMP synthase	6.3.4.1	ATP	7.28E-04	9.63E-03
biotin carboxylase	6.3.4.14	ATP	1.17E-04	9.63E-03
GMP synthase (glutamine-hydrolysing)	6.3.5.2	ATP	5.30E-04	9.63E-03
asparagine synthase (glutamine-hydrolysing)	6.3.5.4	ATP	1.80E-04	9.63E-03
carbamoyl-phosphate synthase (glutamine-hydrolysing)	6.3.5.5	ATP	7.01E-05	9.63E-03
dihydroorotase	3.5.2.3	carbamoyl-aspartate	1.35E-03	5.90E-04
aconitate hydratase	4.2.1.3	cis-aconitate	3.05E-05	1.61E-05
citrate (pro-3S)-lyase	4.1.3.6	citrate	1.60E-04	2.19E-03
aconitate hydratase	4.2.1.3	citrate	3.57E-03	2.19E-03
cytidylate kinase	2.7.4.14	cmp	3.50E-05	3.60E-04
acetaldehyde dehydrogenase	1.2.1.10	CoA	8.94E-06	1.37E-03
homoserine O-succinyltransferase	2.3.1.46	CoA	6.40E-04	1.37E-03
formate C-acetyltransferase	2.3.1.54	CoA	6.80E-06	1.37E-03
O-succinylbenzoate-CoA ligase	6.2.1.26	CoA	3.60E-04	1.37E-03
long-chain-fatty-acid-CoA ligase	6.2.1.3	CoA	3.98E-05	1.37E-03
tRNA cytidyltransferase	2.7.7.21	CTP	4.39E-05	2.73E-03
3-deoxy-manno-octulosonate cytidyltransferase	2.7.7.38	CTP	2.21E-04	2.73E-03
2-C-methyl-D-erythritol 4-phosphate cytidyltransferase	2.7.7.60	CTP	6.53E-05	2.73E-03

phosphopantothenate-cysteine ligase	6.3.2.5	CTP	2.90E-04	2.73E-03
cytidine deaminase	3.5.4.5	cytidine	2.42E-04	2.59E-06
cytosine deaminase	3.5.4.1	cytosine	2.06E-04	1.41E-05
5'-nucleotidase	3.1.3.5	damp	1.20E-05	8.84E-06
purine-nucleoside phosphorylase	2.4.2.1	deoxyguanosine	4.40E-05	5.22E-07
phosphopentomutase	5.4.2.7	deoxyribose 1-phosphate	1.57E-05	3.03E-04
deoxyribose-phosphate aldolase	4.1.2.4	deoxyribose-5-phosphate	6.40E-04	3.03E-04
glucosamine-1-phosphate N-acetyltransferase	2.3.1.157	glucosamine 1-phosphate	1.50E-04	1.15E-03
T2-induced deoxynucleotide kinase	2.7.4.12	dGMP	8.20E-05	5.07E-05
5'-nucleotidase	3.1.3.5	dGMP	4.70E-05	5.07E-05
glycerol-3-phosphate dehydrogenase [NAD(P)+]	1.1.1.94	DHAP	1.70E-04	3.74E-04
methylglyoxal synthase	4.2.3.3	DHAP	4.13E-04	3.74E-04
dihydroorotate oxidase	1.3.3.1	dihydroorotate	2.94E-05	1.19E-05
dihydroorotase	3.5.2.3	dihydroorotate	7.80E-05	1.19E-05
T2-induced deoxynucleotide kinase	2.7.4.12	dTTP	2.78E-04	4.62E-03
glucose-1-phosphate thymidyltransferase	2.7.7.24	dTTP	2.05E-05	4.62E-03
malate oxidase	1.1.3.3	FAD	1.34E-06	1.73E-04
FMN reductase	1.5.1.29	FAD	1.32E-06	1.73E-04
fructose-bisphosphatase	3.1.3.11	FBP	6.00E-06	1.52E-02
Fructose-bisphosphate aldolase	4.1.2.13	FBP	1.75E-04	1.52E-02
FMN reductase	1.5.1.29	FMN	1.52E-06	5.37E-05
transketolase	2.2.1.1	fructose 6-phosphate	1.10E-03	8.75E-03
transaldolase	2.2.1.2	fructose 6-phosphate	1.11E-03	8.75E-03
glutamine-fructose-6-phosphate transaminase (isomerizing)	2.6.1.16	fructose 6-phosphate	2.00E-03	8.75E-03
6-phosphofructokinase	2.7.1.11	fructose 6-phosphate	1.24E-04	8.75E-03
6-phosphofructokinase	2.7.1.11	fructose 6-phosphate	1.24E-04	8.75E-03
glucosamine-6-phosphate deaminase	3.5.99.6	fructose 6-phosphate	1.70E-03	8.75E-03
mannose-6-phosphate isomerase	5.3.1.8	fructose 6-phosphate	1.50E-04	8.75E-03
glucose-6-phosphate isomerase	5.3.1.9	fructose 6-phosphate	1.71E-04	8.75E-03
succinate dehydrogenase (ubiquinone)	1.3.5.1	fumarate	4.20E-04	1.15E-04
fumarate hydratase	4.2.1.2	fumarate	4.94E-04	1.15E-04
gluconokinase	2.7.1.12	gluconate	2.90E-05	4.16E-05
phosphoglucosamine mutase	5.4.2.10	glucosamine-1-p	8.00E-05	1.15E-03

glucosamine-6-phosphate deaminase	3.5.99.6	glucosamine-6-p	4.96E-04	1.15E-03
phosphoglucosamine mutase	5.4.2.10	glucosamine-6-p	5.00E-05	1.15E-03
UDP-glucose-hexose-1-phosphate uridylyltransferase	2.7.7.12	glucose-1-phosphate	2.65E-04	8.75E-03
glucose-1-phosphate thymidylyltransferase	2.7.7.24	glucose-1-phosphate	3.40E-05	8.75E-03
glucose-1-phosphate adenylyltransferase	2.7.7.27	glucose-1-phosphate	1.42E-04	8.75E-03
UTP-glucose-1-phosphate uridylyltransferase	2.7.7.9	glucose-1-phosphate	1.00E-05	8.75E-03
glucose-1-phosphatase	3.1.3.10	glucose-1-phosphate	4.42E-04	8.75E-03
alpha,alpha-trehalose-phosphate synthase	2.4.1.15	glucose-6-phosphate	5.27E-03	8.75E-03
glucose-6-phosphate isomerase	5.3.1.9	glucose-6-phosphate	2.80E-04	8.75E-03
glutamate dehydrogenase (NADP+)	1.4.1.4	glutamate	1.87E-03	9.60E-02
aspartate transaminase	2.6.1.1	glutamate	1.50E-02	9.60E-02
succinyldiaminopimelate transaminase	2.6.1.17	glutamate	2.51E-03	9.60E-02
aromatic-amino-acid transaminase	2.6.1.57	glutamate	2.80E-04	9.60E-02
glutamate decarboxylase	4.1.1.15	glutamate	5.00E-04	9.60E-02
glutamate-tRNA ligase	6.1.1.17	glutamate	1.59E-04	9.60E-02
glutamate-ammonia ligase	6.3.1.2	glutamate	3.30E-03	9.60E-02
dihydrofolate synthase	6.3.2.12	glutamate	3.90E-03	9.60E-02
tetrahydrofolate synthase	6.3.2.17	glutamate	3.33E-04	9.60E-02
glutamate-cysteine ligase	6.3.2.2	glutamate	7.25E-04	9.60E-02
glutamate synthase (NADPH)	1.4.1.13	glutamine	2.50E-04	3.81E-03
amidophosphoribosyltransferase	2.4.2.14	glutamine	2.98E-03	3.81E-03
glutamine-fructose-6-phosphate transaminase (isomerizing)	2.6.1.16	glutamine	5.10E-04	3.81E-03
glutaminase	3.5.1.2	glutamine	4.20E-04	3.81E-03
anthranilate synthase	4.1.3.27	glutamine	3.60E-04	3.81E-03
glutamine-tRNA ligase	6.1.1.18	glutamine	1.69E-04	3.81E-03
GMP synthase	6.3.4.1	glutamine	1.00E-03	3.81E-03
phosphoribosylformylglycinamidine synthase	6.3.5.3	glutamine	3.00E-05	3.81E-03
asparagine synthase (glutamine-hydrolysing)	6.3.5.4	glutamine	8.92E-04	3.81E-03
carbamoyl-phosphate synthase (glutamine- hydrolysing)	6.3.5.5	glutamine	2.03E-04	3.81E-03
glutathione-disulfide reductase	1.8.1.7	glutathione oxidized	6.80E-05	2.37E-03
glycerol-3-phosphate dehydrogenase	1.1.99.5	glycerol 3-phosphate	3.00E-04	4.90E-05

glycerol-3-phosphate dehydrogenase [NAD(P)+]	1.1.1.94	glycerol-3-phosphate	2.10E-04	4.90E-05
glycerol-3-phosphate O-acyltransferase	2.3.1.15	glycerol-3-phosphate	3.91E-04	4.90E-05
CDP-diacylglycerol-glycerol-3-phosphate 3-phosphatidyltransferase	2.7.8.5	glycerol-3-phosphate	3.20E-04	4.90E-05
GMP reductase	1.7.1.7	GMP	9.60E-05	2.37E-05
5'-nucleotidase	3.1.3.5	GMP	5.10E-04	2.37E-05
GTP cyclohydrolase II	3.5.4.25	GTP	3.29E-04	4.87E-03
guanylate cyclase	4.6.1.2	GTP	7.00E-05	4.87E-03
adenylosuccinate synthase	6.3.4.4	GTP	2.00E-05	4.87E-03
xanthine phosphoribosyltransferase	2.4.2.22	guanine	4.30E-06	1.88E-04
hypoxanthine phosphoribosyltransferase	2.4.2.8	guanine	3.70E-05	1.88E-04
guanine deaminase	3.5.4.3	guanine	1.50E-05	1.88E-04
purine-nucleoside phosphorylase	2.4.2.1	guanosine	2.08E-05	1.62E-06
inosine kinase	2.7.1.73	guanosine	6.10E-06	1.62E-06
histidine-tRNA ligase	6.1.1.21	histidine	1.55E-05	6.76E-05
methionine synthase	2.1.1.13	homocysteine	3.32E-05	3.70E-04
5-methyltetrahydropteroyltriglutamate-homocysteine S-methyltransferase	2.1.1.14	homocysteine	6.90E-05	3.70E-04
IMP dehydrogenase	1.1.1.205	IMP	1.31E-05	2.72E-04
5'-nucleotidase	3.1.3.5	IMP	6.60E-04	2.72E-04
adenylosuccinate synthase	6.3.4.4	IMP	2.66E-05	2.72E-04
branched-chain-amino-acid transaminase	2.6.1.42	isoleucine	8.39E-04	3.03E-04
Isoleucine-tRNA ligase	6.1.1.5	isoleucine	2.90E-05	3.03E-04
phosphoglycerate dehydrogenase	1.1.1.95	ketoglutarate	6.08E-05	4.43E-04
taurine dioxygenase	1.14.11.17	ketoglutarate	1.10E-05	4.43E-04
oxoglutarate dehydrogenase (succinyl-transferring)	1.2.4.2	ketoglutarate	1.00E-04	4.43E-04
oxoglutarate dehydrogenase (succinyl-transferring)	1.2.4.2	ketoglutarate	1.00E-04	4.43E-04
glutamate synthase (NADPH)	1.4.1.13	ketoglutarate	5.86E-06	4.43E-04
glutamate dehydrogenase (NADP+)	1.4.1.4	ketoglutarate	7.47E-04	4.43E-04
2-succinyl-6-hydroxy-2,4-cyclohexadiene-1-carboxylate synthase	2.5.1.64	ketoglutarate	1.71E-05	4.43E-04
aspartate transaminase	2.6.1.1	ketoglutarate	2.47E-04	4.43E-04
acetylornithine transaminase	2.6.1.11	ketoglutarate	2.50E-03	4.43E-04
4-aminobutyrate transaminase	2.6.1.19	ketoglutarate	1.07E-03	4.43E-04

branched-chain-amino-acid transaminase	2.6.1.42	ketoglutarate	1.02E-03	4.43E-04
aromatic-amino-acid transaminase	2.6.1.57	ketoglutarate	1.06E-03	4.43E-04
putrescine aminotransferase	2.6.1.82	ketoglutarate	1.90E-02	4.43E-04
branched-chain-amino-acid transaminase	2.6.1.42	leucine	1.41E-03	3.03E-04
leucine-tRNA ligase	6.1.1.4	leucine	1.97E-05	3.03E-04
lysine decarboxylase	4.1.1.18	lysine	1.73E-03	4.05E-04
lysine-tRNA ligase	6.1.1.6	lysine	3.97E-06	4.05E-04
malate dehydrogenase	1.1.1.37	malate	2.60E-03	1.68E-03
malate dehydrogenase (oxaloacetate-decarboxylating)	1.1.1.38	malate	5.04E-04	1.68E-03
fumarate hydratase	4.2.1.2	malate	4.24E-04	1.68E-03
malate oxidase	1.1.3.3	malate	1.20E-03	1.68E-03
[acyl-carrier-protein] S-malonyltransferase	2.3.1.39	malonyl-CoA	6.89E-05	3.54E-05
methionine-tRNA ligase	6.1.1.10	methionine	9.20E-05	1.45E-04
methionine adenosyltransferase	2.5.1.6	metionine	1.10E-04	1.45E-04
glucosamine-1-phosphate N-acetyltransferase	2.3.1.157	N-acetyl-D-glucosamine 1-phosphate	7.00E-05	8.19E-05
UDP-N-acetylglucosamine diphosphorylase	2.7.7.23	N-acetyl-glucosamine 1-phosphate	7.00E-05	8.19E-05
N-acetylglucosamine-6-phosphate deacetylase	3.5.1.25	N-acetyl-glucosamine 6-P	5.66E-04	8.19E-05
L-threonine 3-dehydrogenase	1.1.1.103	NAD+	1.10E-04	5.35E-02
sorbitol-6-phosphate 2-dehydrogenase	1.1.1.140	NAD+	2.00E-04	5.35E-02
mannitol-1-phosphate 5-dehydrogenase	1.1.1.17	NAD+	2.00E-04	5.35E-02
IMP dehydrogenase	1.1.1.205	NAD+	1.70E-04	5.35E-02
UDP-glucose 6-dehydrogenase	1.1.1.22	NAD+	5.00E-05	5.35E-02
histidinol dehydrogenase	1.1.1.23	NAD+	5.70E-04	5.35E-02
shikimate dehydrogenase	1.1.1.25	NAD+	3.88E-05	5.35E-02
quininate/shikimate dehydrogenase	1.1.1.282	NAD+	1.50E-05	5.35E-02
malate dehydrogenase	1.1.1.37	NAD+	2.60E-04	5.35E-02
malate dehydrogenase (oxaloacetate-decarboxylating)	1.1.1.38	NAD+	5.51E-05	5.35E-02
tagaturonate reductase	1.1.1.58	NAD+	8.99E-05	5.35E-02
(R)-aminopropanol dehydrogenase	1.1.1.75	NAD+	1.41E-04	5.35E-02
3-isopropylmalate dehydrogenase	1.1.1.85	NAD+	3.21E-04	5.35E-02
phosphoglycerate dehydrogenase	1.1.1.95	NAD+	7.90E-06	5.35E-02

acetaldehyde dehydrogenase	1.2.1.10	NAD ⁺	6.32E-05	5.35E-02
glyceraldehyde-3-phosphate dehydrogenase (phosphorylating)	1.2.1.12	NAD ⁺	4.50E-05	5.35E-02
aminobutyraldehyde dehydrogenase	1.2.1.19	NAD ⁺	4.46E-05	5.35E-02
lactaldehyde dehydrogenase	1.2.1.22	NAD ⁺	1.83E-04	5.35E-02
phenylacetaldehyde dehydrogenase	1.2.1.39	NAD ⁺	3.50E-05	5.35E-02
erythrose-4-phosphate dehydrogenase	1.2.1.72	NAD ⁺	2.43E-04	5.35E-02
betaine-aldehyde dehydrogenase	1.2.1.8	NAD ⁺	2.67E-05	5.35E-02
prephenate dehydrogenase	1.3.1.12	NAD ⁺	1.64E-04	5.35E-02
dihydrolipoyl dehydrogenase	1.8.1.4	NAD ⁺	4.79E-04	5.35E-02
NAD ⁺ kinase	2.7.1.23	NAD ⁺	1.92E-03	5.35E-02
nicotinamide-nucleotide adenylyltransferase	2.7.7.1	NAD ⁺	3.70E-04	5.35E-02
DNA ligase	6.5.1.2	NAD ⁺	4.58E-07	5.35E-02
sorbitol-6-phosphate 2-dehydrogenase	1.1.1.140	NADH	2.50E-05	4.73E-04
malate dehydrogenase	1.1.1.37	NADH	6.10E-05	4.73E-04
tagaturonate reductase	1.1.1.58	NADH	7.14E-05	4.73E-04
ketol-acid reductoisomerase	1.1.1.86	NADH	2.07E-04	4.73E-04
nitric oxide dioxygenase	1.14.12.17	NADH	3.92E-06	4.73E-04
acetaldehyde dehydrogenase	1.2.1.10	NADH	5.00E-05	4.73E-04
dihydrodipicolinate reductase	1.3.1.26	NADH	3.25E-06	4.73E-04
enoyl-[acyl-carrier-protein] reductase (NADH)	1.3.1.9	NADH	2.00E-05	4.73E-04
pyrroline-5-carboxylate reductase	1.5.1.2	NADH	2.30E-04	4.73E-04
methylenetetrahydrofolate reductase [NAD(P)H]	1.5.1.20	NADH	1.84E-05	4.73E-04
FMN reductase	1.5.1.29	NADH	4.43E-05	4.73E-04
6,7-dihydropteridine reductase	1.5.1.34	NADH	9.10E-05	4.73E-04
NAD(P) ⁺ transhydrogenase (AB-specific)	1.6.1.2	NADH	1.70E-06	4.73E-04
NAD(P)H dehydrogenase (quinone)	1.6.5.2	NADH	1.32E-04	4.73E-04
NADH dehydrogenase (quinone)	1.6.99.5	NADH	5.51E-05	4.73E-04
nitrite reductase [NAD(P)H]	1.7.1.4	NADH	1.60E-05	4.73E-04
glutathione-disulfide reductase	1.8.1.7	NADH	3.77E-04	4.73E-04
serine 3-dehydrogenase	1.1.1.276	NADP ⁺	5.40E-04	2.08E-06
UDP-N-acetylmuramate dehydrogenase	1.1.1.158	NADPH	1.26E-05	1.21E-04
2-dehydropantoate 2-reductase	1.1.1.169	NADPH	2.00E-05	1.21E-04
5-amino-6-(5-phosphoribosylamino)uracil reductase	1.1.1.193	NADPH	5.00E-06	1.21E-04

1-deoxy-D-xylulose-5-phosphate reductoisomerase	1.1.1.267	NADPH	1.08E-05	1.21E-04
GDP-L-fucose synthase	1.1.1.271	NADPH	9.00E-06	1.21E-04
homoserine dehydrogenase	1.1.1.3	NADPH	9.00E-05	1.21E-04
ketol-acid reductoisomerase	1.1.1.86	NADPH	3.28E-06	1.21E-04
glycerol-3-phosphate dehydrogenase [NAD(P)+]	1.1.1.94	NADPH	1.00E-05	1.21E-04
nitric oxide dioxygenase	1.14.12.17	NADPH	1.80E-04	1.21E-04
glycolaldehyde dehydrogenase	1.2.1.21	NADPH	6.56E-05	1.21E-04
glutamyl-tRNA reductase	1.2.1.70	NADPH	3.90E-05	1.21E-04
dihydrodipicolinate reductase	1.3.1.26	NADPH	1.65E-05	1.21E-04
2,4-dienoyl-CoA reductase (NADPH)	1.3.1.34	NADPH	5.00E-05	1.21E-04
trans-2-enoyl-CoA reductase (NADPH)	1.3.1.38	NADPH	4.90E-06	1.21E-04
glutamate synthase (NADPH)	1.4.1.13	NADPH	4.12E-06	1.21E-04
glutamate dehydrogenase (NADP+)	1.4.1.4	NADPH	4.88E-05	1.21E-04
pyrroline-5-carboxylate reductase	1.5.1.2	NADPH	3.00E-05	1.21E-04
FMN reductase	1.5.1.29	NADPH	2.74E-05	1.21E-04
dihydrofolate reductase	1.5.1.3	NADPH	3.13E-06	1.21E-04
NAD(P)+ transhydrogenase (AB-specific)	1.6.1.2	NADPH	2.15E-05	1.21E-04
sulfite reductase (NADPH)	1.8.1.2	NADPH	1.93E-05	1.21E-04
glutathione-disulfide reductase	1.8.1.7	NADPH	2.00E-05	1.21E-04
orotate phosphoribosyltransferase	2.4.2.10	OMP	3.10E-06	1.00E-04
orotidine-5'-phosphate decarboxylase	4.1.1.23	OMP	2.45E-06	1.00E-04
ornithine carbamoyltransferase	2.1.3.3	ornithine	8.50E-04	1.01E-05
ornithine decarboxylase	4.1.1.17	ornithine	4.49E-03	1.01E-05
orotate phosphoribosyltransferase	2.4.2.10	orotate	3.00E-05	5.15E-04
3-phosphoshikimate 1-carboxyvinyltransferase	2.5.1.19	PEP	3.74E-05	1.84E-04
3-deoxy-7-phosphoheptulonate synthase	2.5.1.54	PEP	3.21E-05	1.84E-04
3-deoxy-8-phosphooctulonate synthase	2.5.1.55	PEP	5.56E-06	1.84E-04
UDP-N-acetylglucosamine 1-carboxyvinyltransferase	2.5.1.7	PEP	1.28E-06	1.84E-04
phosphoenolpyruvate carboxylase	4.1.1.31	PEP	6.00E-04	1.84E-04
phosphoenolpyruvate carboxykinase	4.1.1.49	PEP	7.00E-05	1.84E-04
phenylalanine-tRNA	6.1.1.20	phenylalanine	2.27E-05	1.82E-05
aromatic-amino-acid transaminase	2.6.1.57	phenylpyruvate	5.60E-05	1.34E-04
proline-tRNA ligase	6.1.1.15	proline	2.26E-04	3.85E-04

2-methylcitrate synthase	2.3.3.5	propionyl-CoA	1.70E-05	5.32E-06
orotate phosphoribosyltransferase	2.4.2.10	PRPP	3.80E-05	2.58E-04
nicotinate phosphoribosyltransferase	2.4.2.11	PRPP	3.00E-05	2.58E-04
amidophosphoribosyltransferase	2.4.2.14	PRPP	5.96E-05	2.58E-04
anthranilate phosphoribosyltransferase	2.4.2.18	PRPP	1.00E-04	2.58E-04
nicotinate-nucleotide diphosphorylase (carboxylating)	2.4.2.19	PRPP	1.56E-05	2.58E-04
xanthine phosphoribosyltransferase	2.4.2.22	PRPP	1.39E-04	2.58E-04
adenine phosphoribosyltransferase	2.4.2.7	PRPP	3.54E-05	2.58E-04
hypoxanthine phosphoribosyltransferase	2.4.2.8	PRPP	3.30E-05	2.58E-04
uracil phosphoribosyltransferase	2.4.2.9	PRPP	1.48E-04	2.58E-04
nicotinate-nucleotide diphosphorylase (carboxylating)	2.4.2.19	quinolinate	6.40E-06	1.15E-05
FMN reductase	1.5.1.29	riboflavin	1.79E-06	1.90E-05
transketolase	2.2.1.1	ribose-5P	1.40E-03	1.32E-03
ribose-phosphate diphosphokinase	2.7.6.1	ribose-5P	1.90E-04	1.32E-03
ribose-5-phosphate isomerase	5.3.1.6	ribose-5P	2.25E-03	1.32E-03
arabinose-5-phosphate isomerase	5.3.1.13	ribulose-5-P	3.18E-04	1.32E-03
[formate-C-acetyltransferase]-activating enzyme	1.97.1.4	SAM	2.80E-06	1.84E-04
methionine synthase	2.1.1.13	SAM	1.02E-06	1.84E-04
cyclopropane-fatty-acyl-phospholipid synthase	2.1.1.79	SAM	8.98E-05	1.84E-04
adenosylmethionine-8-amino-7-oxononanoate transaminase	2.6.1.62	SAM	5.14E-05	1.84E-04
adenosylmethionine decarboxylase	4.1.1.50	SAM	7.75E-05	1.84E-04
serine 3-dehydrogenase	1.1.1.276	serine	4.00E-02	6.80E-05
glycine hydroxymethyltransferase	2.1.2.1	serine	2.12E-04	6.80E-05
serine O-acetyltransferase	2.3.1.30	serine	1.10E-04	6.80E-05
CDP-diacylglycerol-serine O- phosphatidyltransferase	2.7.8.8	serine	8.50E-05	6.80E-05
tryptophan synthase	4.2.1.20	serine	3.40E-04	6.80E-05
L-serine ammonia-lyase	4.3.1.17	serine	1.75E-02	6.80E-05
Serine-tRNA ligase	6.1.1.11	serine	2.15E-05	6.80E-05
shikimate kinase	2.7.1.71	shikimate	5.85E-04	1.41E-05
succinate dehydrogenase (ubiquinone)	1.3.5.1	succinate	7.07E-05	5.69E-04

2,3,4,5-tetrahydropyridine-2,6-dicarboxylate N-succinyltransferase	2.3.1.117	succinyl-CoA	1.50E-05	2.33E-04
homoserine O-succinyltransferase	2.3.1.46	succinyl-CoA	1.70E-04	2.33E-04
L-threonine 3-dehydrogenase	1.1.1.103	threonine	1.05E-03	1.79E-04
threonine aldolase	4.1.2.5	threonine	2.85E-03	1.79E-04
threonine ammonia-lyase	4.3.1.19	threonine	4.12E-02	1.79E-04
threonine-tRNA ligase	6.1.1.3	threonine	1.34E-04	1.79E-04
tryptophanase	4.1.99.1	tryptophan	3.81E-04	1.21E-05
tryptophan-tRNA ligase	6.1.1.2	tryptophan	1.70E-05	1.21E-05
tyrosine-tRNA ligase	6.1.1.1	tyrosine	6.40E-06	2.89E-05
ribonucleoside-diphosphate reductase	1.17.4.1	UDP	2.20E-04	1.79E-03
UDP-glucose 6-dehydrogenase	1.1.1.22	UDP-glucose	2.57E-03	2.50E-03
alpha,alpha-trehalose-phosphate synthase	2.4.1.15	UDP-glucose	6.62E-03	2.50E-03
lipopolysaccharide glucosyltransferase I	2.4.1.58	UDP-glucose	1.90E-04	2.50E-03
lipopolysaccharide glucosyltransferase II	2.4.1.73	UDP-glucose	9.00E-06	2.50E-03
UDP-glucose-hexose-1-phosphate uridylyltransferase	2.7.7.12	UDP-glucose	2.00E-04	2.50E-03
UDP-sugar diphosphatase	3.6.1.45	UDP-glucose	2.00E-05	2.50E-03
UDP-glucose 4-epimerase	5.1.3.2	UDP-glucose	1.20E-03	2.50E-03
acyl-[acyl-carrier-protein]-UDP-N-acetylglucosamine O-acyltransferase	2.3.1.129	UDP-N-ac-glucosamine	2.85E-04	9.24E-03
undecaprenyldiphospho-muramoylpentapeptide beta-N-acetylglucosaminyltransferase	2.4.1.227	UDP-N-ac-glucosamine	6.25E-05	9.24E-03
UDP-N-acetylglucosamine 1-carboxyvinyltransferase	2.5.1.7	UDP-N-ac-glucosamine	5.98E-05	9.24E-03
UDP-N-acetylglucosamine 2-epimerase	5.1.3.14	UDP-N-ac-glucosamine	6.15E-04	9.24E-03
uridine kinase	2.7.1.48	uridine	3.50E-04	2.09E-03
glucosamine-1-phosphate N-acetyltransferase	2.3.1.157	UTP	1.00E-04	8.29E-03
UDP-N-acetylglucosamine diphosphorylase	2.7.7.23	UTP	1.00E-04	8.29E-03
UTP-glucose-1-phosphate uridylyltransferase	2.7.7.9	UTP	7.00E-05	8.29E-03
CTP synthase	6.3.4.2	UTP	4.38E-05	8.29E-03
branched-chain-amino-acid transaminase	2.6.1.42	valine	2.91E-03	4.02E-03
valine-tRNA ligase	6.1.1.9	valine	2.57E-05	4.02E-03

Supplementary Table 8: Compounds measured to determine completeness of labeling

Labeling group	Measured compounds
Glycolysis	FBP, DHAP, phenylalanine, valine, alanine, serine, tryptophan, histidine
Aspartate	aconitate, α -ketoglutarate, malate, citrate, aspartate, glutamate, methionine, proline, threonine
Purine	riboflavin, ADP, ATP
pyrimidines	CTP, UDP-glucose, UDP-glucuronate, UDP-N-acetyl-glucosamine
NAD	NAD ⁺ , NADP ⁺ , SAM
succinate/fumarate	succinate

Supplementary Methods

Liquid chromatography-tandem mass spectrometry

The LC method coupled to positive mode ESI was hydrophilic interaction chromatography on an aminopropyl column at basic pH as previously reported³. The LC method coupled to negative mode ESI was reversed phase chromatography with an amine-based ion pairing agent⁴ with only the LC gradient modified to: $t = 0$, 0% B; $t = 5$, 0% B; $t = 10$, 20% B; $t = 20$, 20% B; $t = 35$, 65% B; $t = 38$, 95% B; $t = 42$, 95% B, $t = 43$, 0% B; $t = 50$, 0% B where B refers to the methanol-containing mobile phase. LC instrumentation was an LC-20 AD HPLC system (Shimadzu), autosampler temperature of 4°C, injection volume 10 μ L.

Media and culture conditions

Complete media formulation was 4.7g/L KH_2PO_4 , 13.5g/L K_2HPO_4 , 1 g/L K_2SO_4 , 0.1 g/L $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, and final concentrations of 10 mM NH_4Cl , and 4g/L of the carbon source (acetate was added as 6.65 g/L of potassium acetate). Agarose plates for filter cultures were prepared by mixing triply-washed agarose with the above media composition to a final concentration of 1.5% agarose, and then pouring the mixture (~30 mL/plate) into 10 cm Petri dishes

In order to prepare filter cultures, a single colony was picked from a Luria Broth plate, and grown to saturation overnight in minimal media (containing either glucose, or, for glycerol and acetate cultures, a 1:4 mass ratio of glucose to the other carbon source). The saturated overnight culture was then diluted to OD_{650} (optical density at 650 nm) of 0.03 into liquid minimal medium with glucose, glycerol, or acetate as the sole carbon source. This liquid culture was grown to OD_{650} of ~ 0.1, at which time it was transferred to filter culture as follows: for each filter culture, 5 mL of liquid culture was passed through an 82 mm diameter round nylon filter (GE cat# N00HY08250) and the filter placed cell-side up onto a medium-loaded agarose plate. The filter cultures were allowed to grow to an OD_{650} of 0.35 (determined by washing a filter in 5 mL of liquid medium, then determining OD_{650}) prior to quenching metabolism and extracting metabolites.

Preparation of internal standards

Metabolite standards were purchased from Sigma-Aldrich in the highest purity available. A stock solution of each metabolite standard was prepared by dissolving the standard in 100% acetonitrile to a concentration of 100-2000 ng/mL. The stock solutions were stored at -80°C and used within 3 days of preparation.

Metabolites were quantified in batches of 15-20 compounds. For each batch, the extraction solution containing the 15-20 internal standards was prepared by mixing the

standard stock solutions to obtain final concentrations of each standard as close as possible to the corresponding endogenous metabolite concentration, with solvent added to obtain a final composition of 40:40:20 acetonitrile:methanol:water with 0.1 M formic acid. The extraction solution was maintained at -20°C for ~ 2 h before extracting cells.

For each batch of internal standards, an initial experiment was performed in order to determine the appropriate concentration of internal standard. Extraction solution was prepared containing each of 15-20 internal standards at a final concentration of ~1-10 nM in 40:40:20 acetonitrile:methanol:water with 0.1 M formic and used to extract two filter cultures grown with U-¹³C-glucose as the carbon source. Peak heights of the standards (unlabelled) and endogenous metabolites (¹³C-labeled) were compared. If the standard and endogenous peak heights differed by more than 5-fold, changes in dilution were calculated to make the peak heights match more closely. Subsequent to determining the appropriate concentration for each internal standard, a new extraction solution of appropriate concentrations was prepared.

Quantitation of glycerol- and acetate-grown cells

Concentrations were determined based on the ratio of the ¹²C peak height (from the glycerol- or acetate-fed culture) and the ¹³C-peak height (from the cellular ¹³C-labeled internal standard from ¹³C-glucose-fed cells), along with the other values determined during metabolite quantification on glucose. Calculation of the concentration was according to the equation:

$$C_{avg} = R \times K \times S \times V_1 \times \frac{DW_{cell}}{DW_{tot} \times V_{cell}}$$

where all values are identical to those used for calculating glucose concentration (see main text), except for *K*, which is the geometric mean ratio of the ¹²C-peak height (corrected for naturally occurring ¹³C) to the ¹³C-peak height. Error was determined by propagation of uncertainty in *R* and *K* as standard errors in logarithmic space as described for glucose-grown cultures in the main text.

Correction for excreted metabolites

In order to correct the data for metabolites that had been excreted during growth, filter cultures were grown in unlabelled glucose, glycerol, or acetate. Each of the filter cultures was extracted as described above. Additionally, immediately after removal of each cell-loaded filter from the agarose plate, a fresh empty filter was placed on the plate and allowed to equilibrate for 60 seconds (thereby absorbing extracellular solution equivalent to that present surrounding the cells on the cell-loaded filter). These filters were extracted in the same manner as the filter cultures. Each sample was then mixed 1:1 with cellular ¹³C-labeled internal standard. The fraction of each metabolite that was extracellular was determined as the ratio of the ¹²C- to ¹³C-signal from the cell-free extraction to the ¹²C- to ¹³C-signal from the cell-containing extract. Intracellular

concentrations were corrected for the amount of metabolite found to be extracellular by multiplying the originally calculated concentration by the fraction of the total signal that was intracellular.

Tests of statistical significance

In order to determine which metabolite concentrations were significantly different between cells grown in the three carbon sources, we performed a two-tailed Student's t-test. Note that uncertainty in the measurement of R does not contribute to uncertainty in relative concentrations (although it does contribute to uncertainty in absolute cellular concentrations) and was accordingly not included in the error estimates entered into the t-tests. False discovery rate was determined from the p-values using the approach of Benjamini and Hochberg⁵ and differences significant at a false discovery rate of less than 0.05 were deemed significant. For testing enrichment, we used the hypergeometric test implemented in the statistical software R .

Michaelis constants

Michaelis constants were obtained from the BRENDA database⁶. Only values for the K_m of compounds annotated as substrates for the forward reactions in Ecocyc⁷ were included in the analysis. In the case that there were divergent values of K_m reported in BRENDA, the geometric mean of all available values was used in the analysis. The full data set is available (**Supplementary Table 7**).

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