

Supplementary Information Appendix

Enhancing a multipurpose artificial urine for culture and gene expression studies of uropathogenic *Escherichia coli* strains

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Tables S1-S4

Figure S1

Table S1. Supplements added to MP-AU to prepare enhanced AU. Supplements were added at a final concentration of 0.5 mM.

Component	Supplier
D-alanine	Sigma-Aldrich
D-serine	
D-sorbitol	
Glycine	
Hippuric acid	
L-alanine	
L-arginine	
L-aspartic acid	
L-carnitine	
L-citrulline	
L-cysteine hydrochloride	
L-glutamic acid monosodium salt hydrate	
L-glutamine	
L-histidine	
L-lysine	
L-methionine	
L-ornithine monohydrochloride	
L-proline	
L-serine	
L-tryptophan	
L-tyrosine	

Table S2. Abundance of metabolites identified in pooled human urine vs multi-purpose artificial urine.

Name	Formula	Log fold change
N-Acetylglutamine	C ₇ H ₁₂ N ₂ O ₄	12.00
L-Citrulline	C ₆ H ₁₃ N ₃ O ₃	10.85
L-Carnitine	C ₇ H ₁₅ NO ₃	10.77
N(pi)-Methyl-L-histidine	C ₇ H ₁₁ N ₃ O ₂	10.68
Glycine	C ₂ H ₅ NO ₂	10.47
L-Tryptophan	C ₁₁ H ₁₂ N ₂ O ₂	10.03
D-Galacturonate	C ₆ H ₁₀ O ₇	9.66
L-Glutamine	C ₅ H ₁₀ N ₂ O ₃	9.36
D-Threose	C ₄ H ₈ O ₄	9.26
L-Tyrosine	C ₉ H ₁₁ NO ₃	9.24
D-Ribose	C ₅ H ₁₀ O ₅	9.20
O-Acetylcarnitine	C ₉ H ₁₇ NO ₄	9.16
2-Oxoglutarate	C ₅ H ₆ O ₅	9.11
Ala-Gly	C ₅ H ₁₀ N ₂ O ₃	9.10
sucrose	C ₁₂ H ₂₂ O ₁₁	9.10
Betaine	C ₅ H ₁₁ NO ₂	8.56
N2-Acetyl-L-lysine	C ₈ H ₁₆ N ₂ O ₃	8.45
L-Serine	C ₃ H ₇ NO ₃	8.42
N-Acetylneuraminate	C ₁₁ H ₁₉ NO ₉	8.39
Hypoxanthine	C ₅ H ₄ N ₄ O	8.39
Pantothenate	C ₉ H ₁₇ NO ₅	8.27
D-Gluconic acid	C ₆ H ₁₂ O ₇	8.25
N-acetyl-L-glutamate	C ₇ H ₁₁ NO ₅	8.24
L-Aspartate	C ₄ H ₇ NO ₄	8.22
L-Methionine	C ₅ H ₁₁ NO ₂ S	8.00
5-Oxoproline	C ₅ H ₇ NO ₃	7.86
L-2-Aminoadipate	C ₆ H ₁₁ NO ₄	7.55

L-Kynurenine	$C_{10}H_{12}N_2O_3$	7.52
Choline	$C_5H_{13}NO$	7.46
sn-glycero-3-Phosphocholine	$C_8H_{20}NO_6P$	7.27
Xanthine	$C_5H_4N_4O_2$	7.19
Adenosine	$C_{10}H_{13}N_5O_4$	7.18
D-Galactarate	$C_6H_{10}O_8$	7.10
L-Lysine	$C_6H_{14}N_2O_2$	7.09
Orotidine	$C_{10}H_{12}N_2O_8$	6.94
N-Acetyl-D-glucosamine	$C_8H_{15}NO_6$	6.94
riboflavin	$C_{17}H_{20}N_4O_6$	6.86
L-Gulono-1,4-lactone	$C_6H_{10}O_6$	6.69
L-Cystine	$C_6H_{12}N_2O_4S_2$	6.50
2-Methylcitrate	$C_7H_{10}O_7$	6.50
S-Adenosyl-L-homocysteine	$C_{14}H_{20}N_6O_5S$	6.49
L-Cystathionine	$C_7H_{14}N_2O_4S$	5.98
3',5'-Cyclic AMP	$C_{10}H_{12}N_5O_6P$	5.97
N-Acetylornithine	$C_7H_{14}N_2O_3$	5.95
D-Glucosamine	$C_6H_{13}NO_5$	5.38
2-Phenylglycine	$C_8H_9NO_2$	5.34
D-glucose	$C_6H_{12}O_6$	4.86
4-(beta-Acetylaminoethyl)imidazole	$C_7H_{11}N_3O$	4.79
L-Ornithine	$C_5H_{12}N_2O_2$	4.70
(S)-Malate	$C_4H_6O_5$	4.29
L-Arginine	$C_6H_{14}N_4O_2$	4.23
3-Phospho-D-glycerate	$C_3H_7O_7P$	4.08
Inosine	$C_{10}H_{12}N_4O_5$	3.89
Deoxyadenosine	$C_{10}H_{13}N_5O_3$	3.49
Itaconate	$C_5H_6O_4$	3.46
D-glucose 6-phosphate	$C_6H_{13}O_9P$	3.37

cis-Aconitate	$\text{C}_6\text{H}_6\text{O}_6$	2.49
citrate	$\text{C}_6\text{H}_8\text{O}_7$	1.30
L-Glutamate	$\text{C}_5\text{H}_9\text{NO}_4$	1.27
Oxalate	$\text{C}_2\text{H}_2\text{O}_4$	0.76
Creatinine	$\text{C}_4\text{H}_7\text{N}_3\text{O}$	0.21
Orthophosphate	$\text{H}_3\text{O}_4\text{P}$	-0.22
Nicotinamide	$\text{C}_6\text{H}_6\text{N}_2\text{O}$	-0.27
6-Methylaminopurine	$\text{C}_6\text{H}_7\text{N}_5$	-1.88
AMP	$\text{C}_{10}\text{H}_{14}\text{N}_5\text{O}_7\text{P}$	-2.25

Table S3. 50 most significant differentially expressed genes in CFT073 in enhanced AU vs pooled human urine.

Name	Gene product	Function	Fold change	FDR p-value
<i>thiG</i>	thiazole synthase	Thiamine diphosphate biosynthetic process	158.89	7.56E-40
<i>thiE</i>	thiamine phosphate synthase	Thiamine biosynthetic process, thiamine diphosphate biosynthetic process	179.03	7.70E-39
<i>thiF</i>	thiazole biosynthesis adenylyltransferase ThiF	ATP binding, metal ion binding, nucleotidyltransferase activity, ubiquitin-like modifier activating enzyme activity	230.97	4.64E-36
<i>thiD</i>	bifunctional hydroxymethylpyrimidine kinase/phosphomethylpyrimidine kinase	Phosphorylation, thiamine biosynthetic process, thiamine diphosphate biosynthetic process	87.54	5.85E-34
<i>lacY</i>	lactose permease	Carbohydrate:proton symporter activity	-126.12	1.79E-29
<i>thiH</i>	2-iminoacetate synthase ThiH	Thiamine biosynthetic process	146.37	6.42E-29
<i>C_RS01560</i>	PTS sugar transporter subunit IIA	Not available	-90.41	2.84E-28
<i>tnaB</i>	low affinity tryptophan permease TnaB	Aromatic amino acid transmembrane transporter activity	69.48	5.66E-28
<i>lacZ</i>	beta-galactosidase	Carbohydrate catabolic process	-196.98	3.18E-25
<i>tnaA</i>	tryptophanase	Tryptophanase activity	295.36	1.20E-24
<i>C_RS01555</i>	PTS sugar transporter subunit IIB	Phosphoenolpyruvate-dependent sugar phosphotransferase system	-61.38	1.79E-23
<i>thiC</i>	phosphomethylpyrimidine synthase ThiC	Thiamine biosynthetic process, thiamine diphosphate biosynthetic process	135.21	1.93E-22
<i>thiM</i>	hydroxyethylthiazole kinase	Phosphorylation, thiamine biosynthetic process, thiamine diphosphate biosynthetic process	59.69	2.36E-22
<i>srlB</i>	PTS glucitol/sorbitol transporter subunit IIA	Phosphoenolpyruvate-dependent sugar phosphotransferase system	58.84	5.14E-21
<i>torC</i>	pentaheme c-type cytochrome TorC	Anaerobic respiration	-46.31	4.72E-20
<i>nanA</i>	N-acetylneuraminate lyase	Carbohydrate metabolic process, N-acetylneuraminate catabolic process	-51.54	7.49E-20

<i>btsT</i>	pyruvate/proton symporter BtsT	Cellular response to starvation	-68.15	4.38E-18
<i>lacA</i>	galactoside O-acetyltransferase	Acetyltransferase activity	-30.13	1.25E-17
<i>psuK</i>	pseudouridine kinase	Phosphorylation	-44.63	1.25E-16
<i>bioA</i>	adenosylmethionine--8-amino-7-oxononanoate transaminase	Biotin biosynthetic process	26.59	1.68E-16
<i>nadB</i>	L-aspartate oxidase	NAD biosynthetic process	23.09	2.38E-16
<i>dsdX</i>	D-serine transporter DsdX	D-serine transport	-28.73	3.08E-16
<i>nadA</i>	quinolinate synthase NadA	NAD biosynthetic process	29.70	8.49E-16
<i>bioC</i>	malonyl-ACP O-methyltransferase BioC	Biotin biosynthetic process, methylation	18.26	4.64E-15
<i>srIE</i>	PTS glucitol/sorbitol transporter subunit IIB	Phosphoenolpyruvate-dependent sugar phosphotransferase system	60.96	3.00E-14
<i>bioF</i>	8-amino-7-oxononanoate synthase	Biotin biosynthetic process	14.90	7.64E-14
<i>gutM</i>	transcriptional regulator GutM	Not available	34.26	1.24E-13
<i>srlA</i>	PTS glucitol/sorbitol transporter subunit IIC	Phosphoenolpyruvate-dependent sugar phosphotransferase system	54.82	1.86E-13
<i>srlD</i>	sorbitol-6-phosphate dehydrogenase	Sorbitol-6-phosphate 2-dehydrogenase activity	51.28	2.55E-13
<i>C_RS01550</i>	PTS ascorbate transporter subunit IIC	Phosphoenolpyruvate-dependent sugar phosphotransferase system	-41.66	3.89E-13
<i>bioB</i>	biotin synthase BioB	Biotin biosynthetic process	13.34	7.42E-13
<i>hiuH</i>	hydroxyisourate hydrolase	Purine nucleobase metabolic process	-19.97	1.65E-12
<i>C_RS19900</i>	hypothetical protein	Not available	-23.19	1.65E-12
<i>bioD_1</i>	dethiobiotin synthase	Biotin biosynthetic process	13.03	1.65E-12
<i>dsdA</i>	D-serine ammonia-lyase	D-amino acid metabolic process	-27.81	5.91E-12
<i>zraP</i>	zinc resistance sensor/chaperone ZraP	Not available	12.62	2.08E-11
<i>C_RS11805</i>	DeoR family transcriptional regulator	D-ribose catabolic process	-9.40	3.46E-11
<i>nanE</i>	N-acetylmannosamine-6-phosphate 2-epimerase	Carbohydrate metabolic process, N-acetylmannosamine metabolic process, N-acetylneuraminate catabolic process	-11.48	1.03E-10

<i>uxaC</i>	glucuronate isomerase	Glucuronate catabolic process	-11.71	1.04E-10
<i>ygbJ</i>	NAD(P)-dependent oxidoreductase	Carbohydrate metabolic process, organic acid catabolic process	-15.30	1.13E-10
<i>yegX</i>	glycoside hydrolase family 25 protein	Cell wall macromolecule catabolic process, peptidoglycan catabolic process	12.10	4.51E-10
<i>araD_2</i>	L-ribulose-5-phosphate 4-epimerase	L-arabinose catabolic process to xylulose 5-phosphate	14.27	6.66E-10
<i>galP</i>	galactose/proton symporter	Carbohydrate transport	-11.23	7.08E-10
<i>glpD</i>	glycerol-3-phosphate dehydrogenase	Glycerol-3-phosphate metabolic process	-15.03	7.08E-10
<i>psuG</i>	pseudouridine-5'-phosphate glycosidase	Not available	-29.64	9.87E-10
<i>nanT</i>	sialic acid transporter NanT	Carbohydrate transport	-13.07	1.12E-09
<i>nanK</i>	N-acetylmannosamine kinase	N-acetylmannosamine metabolic process, N-acetylneuraminate catabolic process	-12.39	1.62E-09
<i>sgrT</i>	glucose uptake inhibitor SgrT	Not available	-101.52	2.28E-09
<i>idnD</i>	L-idonate 5-dehydrogenase	L-idonate 5-dehydrogenase activity	-21.06	2.55E-09
<i>astE</i>	succinylglutamate desuccinylase	Arginine catabolic process to glutamate, arginine catabolic process to succinate	12.17	2.83E-09

Table S4. 50 most significant differentially expressed genes in UTI89 in enhanced AU vs pooled human urine.

Name	Gene product	Functional group	Fold change	FDR <i>p</i> -value
<i>tnaA</i>	tryptophanase	Tryptophanase activity	1446.32	3.79E-29
<i>thiC</i>	phosphomethylpyrimidine synthase ThiC	Thiamine biosynthetic process, thiamine diphosphate biosynthetic process	196.09	1.19E-22
<i>thiD</i>	bifunctional hydroxymethylpyrimidine kinase/phosphomethylpyrimidine kinase	Phosphorylation, thiamine biosynthetic process, thiamine diphosphate biosynthetic process	63.66	4.39E-21
<i>thiG</i>	thiazole synthase	Thiamine diphosphate biosynthetic process	179.56	4.39E-21
<i>thiE</i>	thiamine phosphate synthase	Thiamine biosynthetic process, thiamine diphosphate biosynthetic process	171.08	7.93E-21
<i>thiF</i>	thiazole biosynthesis adenylyltransferase ThiF	ATP binding, metal ion binding, nucleotidyltransferase activity, ubiquitin-like modifier activating enzyme activity	187.10	1.76E-20
<i>lacZ</i>	beta-galactosidase	Carbohydrate catabolic process	-191.23	4.11E-19
<i>bioF</i>	8-amino-7-oxononanoate synthase	Biotin biosynthetic process	60.96	6.99E-19
<i>bioA</i>	adenosylmethionine--8-amino-7-oxononanoate transaminase	Biotin biosynthetic process	48.36	6.73E-18
<i>lacY</i>	lactose permease	Carbohydrate:proton symporter activity	-132.55	8.65E-17
<i>thiH</i>	2-iminoacetate synthase ThiH	Thiamine biosynthetic process	107.68	2.05E-16
<i>yegX</i>	glycoside hydrolase family 25 protein	Cell wall macromolecule catabolic process, peptidoglycan catabolic process	62.45	3.98E-16
<i>bioB</i>	biotin synthase BioB	Biotin biosynthetic process	36.95	6.57E-16
<i>bioC</i>	malonyl-ACP O-methyltransferase BioC	Biotin biosynthetic process, methylation	41.93	1.20E-15
<i>tnaB</i>	low affinity tryptophan permease TnaB	Aromatic amino acid transmembrane transporter activity	119.04	4.39E-14
<i>lacA</i>	galactoside O-acetyltransferase	Acetyltransferase activity	-79.66	6.91E-14
<i>UTI89_RS19025</i>	hypothetical protein	Not available	-185.23	2.75E-13

<i>yeaR</i>	DUF1971 domain-containing protein YeaR	Not available	-28.61	5.16E-13
<i>bioD_1</i>	dethiobiotin synthase	Biotin biosynthetic process	24.26	7.99E-13
<i>nanA</i>	N-acetylneuraminate lyase	Carbohydrate metabolic process, N-acetylneuraminate catabolic process	-57.06	1.12E-12
<i>thiM</i>	hydroxyethylthiazole kinase	Thiamine biosynthetic process, thiamine diphosphate biosynthetic process	39.03	1.12E-12
<i>nadA</i>	quinolinate synthase NadA	NAD biosynthetic process	52.50	9.70E-12
<i>sfaD</i>	S-fimbrial adhesin minor subunit SfaS	Cell adhesion	16.80	1.87E-11
<i>thiS</i>	sulfur carrier protein ThiS	Thiamine biosynthetic process	879.87	3.46E-11
<i>torC</i>	pentaheme c-type cytochrome TorC	Anaerobic respiration	-13.11	6.82E-10
<i>UTI89_RS05345</i>	fimbria/pilus periplasmic chaperone	Regulation of DNA-templated transcription	11.76	8.86E-10
<i>galP</i>	galactose/proton symporter	Transmembrane transporter activity	-36.07	1.06E-09
<i>putA</i>	trifunctional transcriptional regulator/proline dehydrogenase/L-glutamate gamma-semialdehyde dehydrogenase	Proline biosynthetic process, proline catabolic process to glutamate	45.79	3.56E-09
<i>UTI89_RS05360</i>	fimbrial protein	Cell wall organisation, chaperone-mediated protein folding	16.87	3.99E-09
<i>UTI89_RS19030</i>	hypothetical protein	Not available	-79.50	6.63E-09
<i>uidB</i>	glucuronide transporter	Carbohydrate transport, sodium ion transport	-46.55	7.75E-09
<i>uidA</i>	beta-glucuronidase	Carbohydrate catabolic process	-53.60	1.68E-08
<i>yoaG</i>	DUF1869 domain-containing protein	Not available	-26.05	2.13E-08
<i>UTI89_RS24120</i>	pyridoxal phosphate-dependent aminotransferase	Biosynthetic process	-37.27	3.00E-08
<i>nanE</i>	N-acetylmannosamine-6-phosphate 2-epimerase	Carbohydrate metabolic process, N-acetylneuraminate metabolic process, N-acetylneuraminate catabolic process	-23.45	3.78E-08
<i>ygjI</i>	amino acid permease	Transmembrane transporter activity	-34.88	4.27E-08

<i>ebgC</i>	beta-galactosidase subunit beta	Metabolic process	-30.65	7.74E-08
<i>nanC</i>	N-acetylneuraminic acid outer membrane channel NanC	Carbohydrate transport	-21.57	2.35E-07
<i>ygbL</i>	aldolase	Monosaccharide metabolic process	-72.54	3.12E-07
<i>idnO</i>	gluconate 5-dehydrogenase	Gluconate 5-dehydrogenase activity	-31.18	3.27E-07
<i>nadB</i>	L-aspartate oxidase	NAD biosynthetic process	17.51	3.47E-07
<i>glpB</i>	glycerol-3-phosphate dehydrogenase subunit GlpB	Glycerol catabolic process, glycerol-3-phosphate metabolic process	-20.08	4.20E-07
<i>btsT</i>	pyruvate/proton symporter BtsT	Cellular response to starvation	-35.12	6.90E-07
<i>idnD</i>	L-idonate 5-dehydrogenase	L-idonate 5-dehydrogenase activity	-33.74	7.16E-07
<i>ygbK</i>	four-carbon acid sugar kinase family protein	Carbohydrate metabolic process, phosphorylation	-58.36	7.16E-07
<i>ygbN</i>	GntP family transporter	Methylation	-72.32	7.16E-07
<i>UTI89_RS22505</i>	CidA/LrgA family protein	Not available	-9.36	7.79E-07
<i>glpD</i>	glycerol-3-phosphate dehydrogenase	Glycerol-3-phosphate metabolic process	-30.35	1.08E-06
<i>nanK</i>	N-acetylmannosamine kinase	N-acetylmannosamine metabolic process, N-acetylneuraminate catabolic process	-17.25	1.08E-06
<i>ygbJ</i>	NAD(P)-dependent oxidoreductase	Organic acid catabolic process	-60.38	1.20E-06

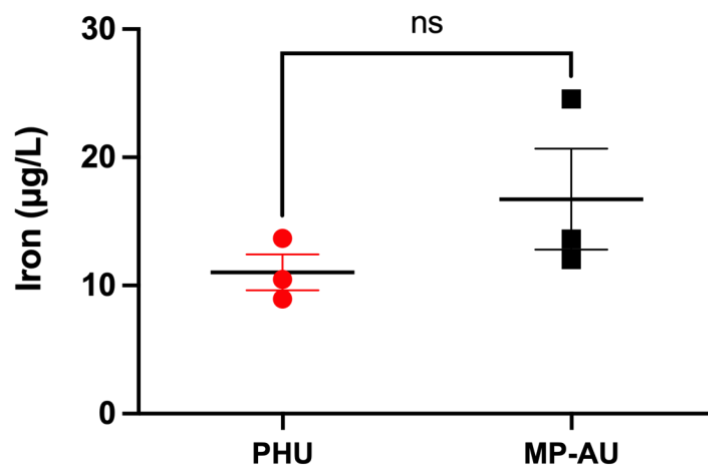


Fig S1. Determining the iron concentration in pooled human urine by inductively-coupled plasma mass spectrometry. The concentration of iron in PHU (red circles) and MP-AU (black squares) was analysed by ICP-MS. Data represent the mean and standard error of the mean of three technical replicates. Statistical significance was determined using Welch's t test.