

Fig. S6. Pairwise scaled epistasis among 270 important *E. coli* reactions in the glucose minimal medium, when mutations are generated by flux constraints up to 50% of the wild-type fluxes. Both rows and columns represent reactions. Reactions 1 to 212 are essential, whereas reactions 213 to 270 are nonessential.

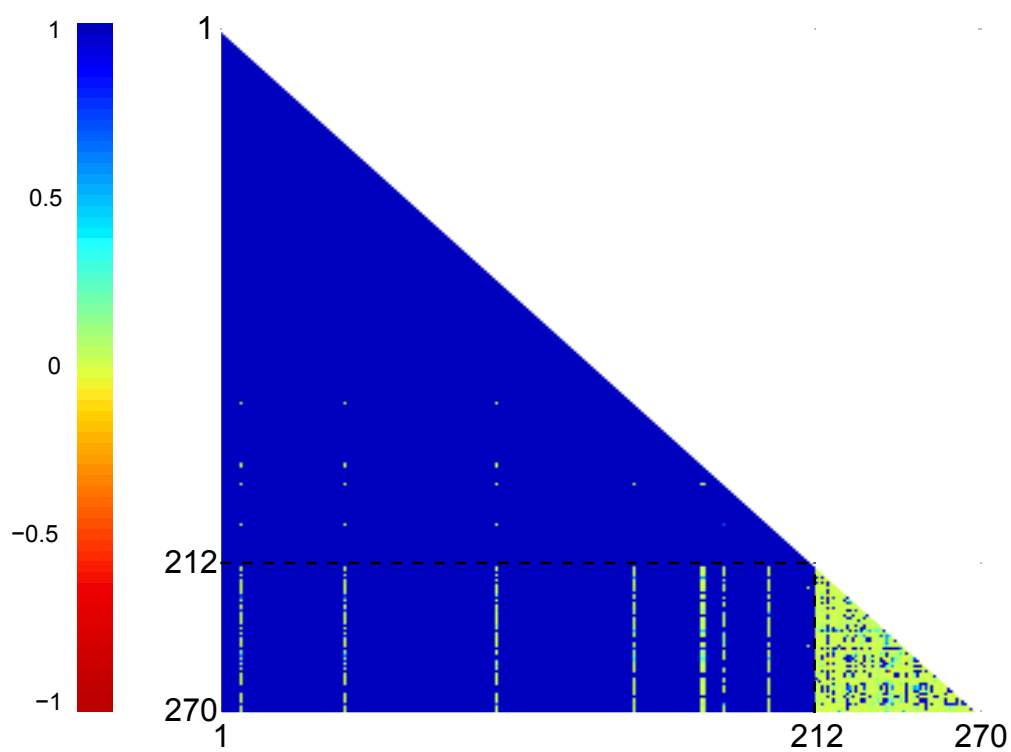


Fig. S7. Pairwise scaled epistasis among 270 important *E. coli* reactions in the glucose minimal medium, when mutations are generated by flux constraints up to 10% of the wild-type fluxes. Both rows and columns represent reactions. Reactions 1 to 212 are essential, whereas reactions 213 to 270 are nonessential.

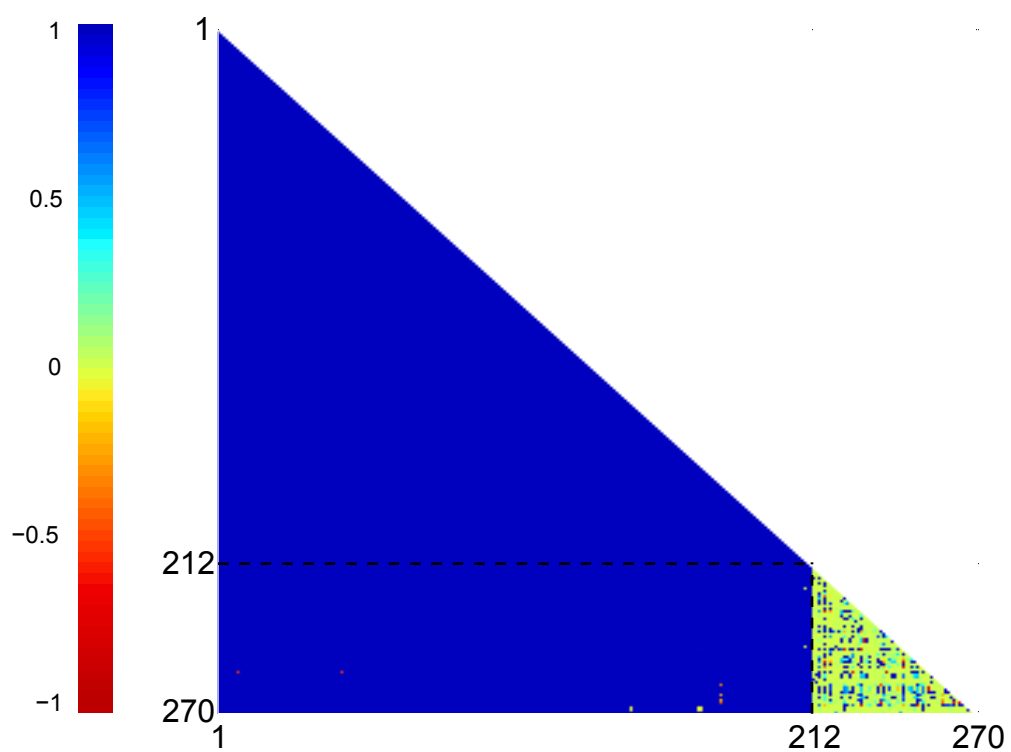


Fig. S8. Pairwise scaled epistasis among 270 important *E. coli* reactions in the glucose minimal medium, when mutations are generated by flux constraints up to 90% of the wild-type fluxes. Both rows and columns represent reactions. Reactions 1 to 212 are essential, whereas reactions 213 to 270 are nonessential.

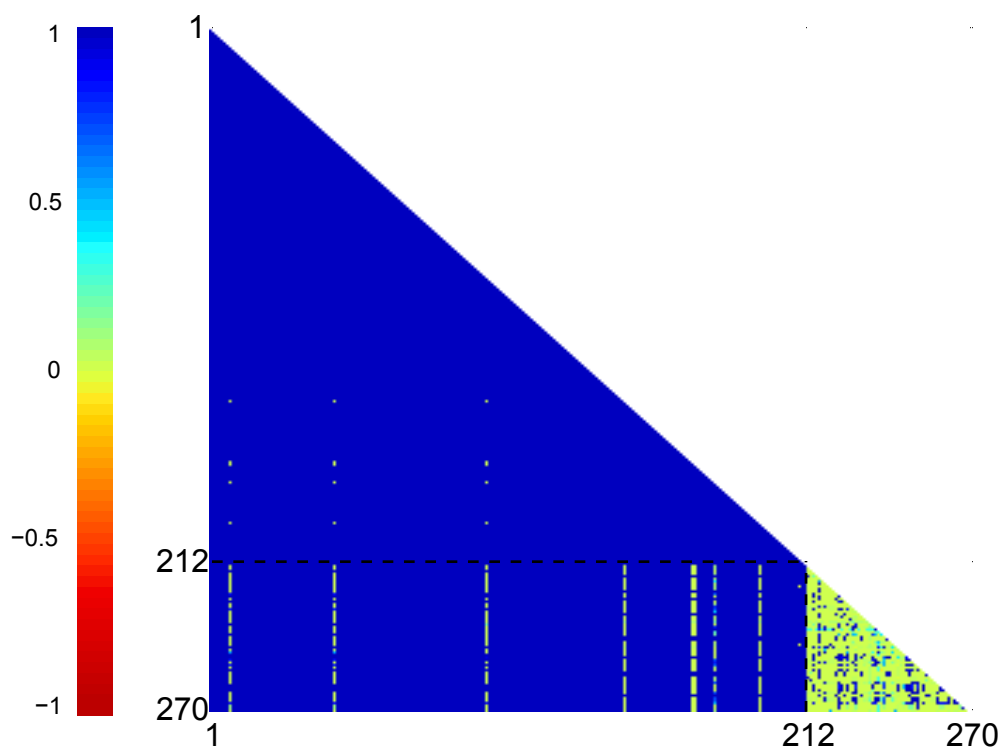


Fig. S9. Functions of 255 *E. coli* metabolic reactions in producing 49 biomass constituents under the acetate minimal medium. The listed reactions are those that are important and have determined functions in the glucose minimal medium, and are listed in the order as they appear in Fig. 1a. Columns represent reactions and rows represent biomass constituents. On the top of the figure, the symbol “a” indicates that the reaction causes no fitness reduction in the acetate minimal medium when deleted (and therefore has undetermined function) and “c” indicates that the function of the reaction in the acetate minimal medium differs from that in the glucose minimal medium.

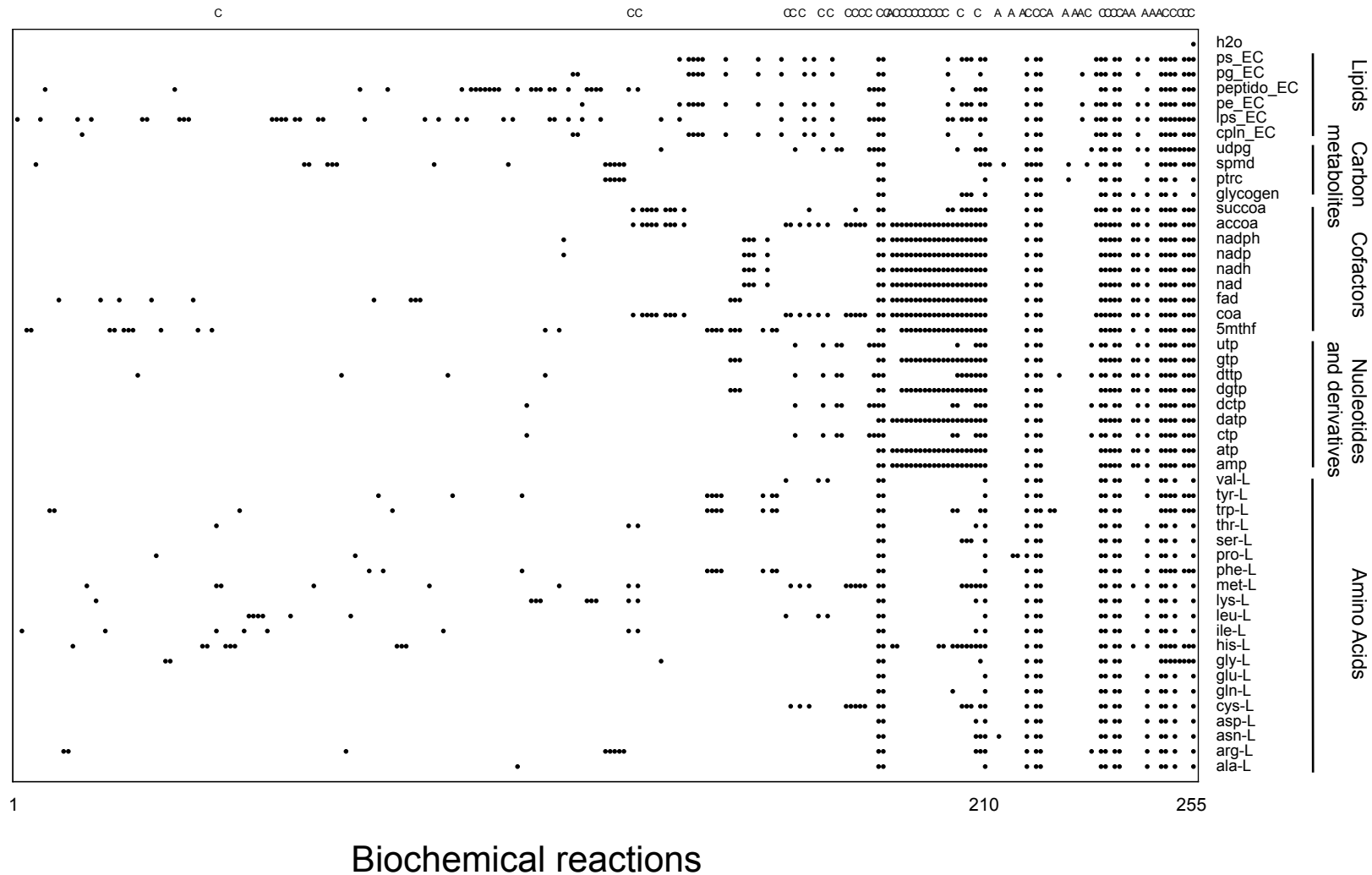


Fig. S10. Functions of 255 of *E. coli* metabolic reactions in producing 49 biomass constituents under the glycerol minimal medium. The listed reactions are those that are important and have determined functions in the glucose minimal medium, and are listed in the order as they appear in Fig. 1a. Columns represent reactions and rows represent biomass constituents. On the top of the figure, the symbol “a” indicates that the reaction causes no fitness reduction in the glycerol minimal medium when deleted (and therefore has undetermined function) and “c” indicates that the function of the reaction in the acetate minimal medium differs from that in the glucose minimal medium.

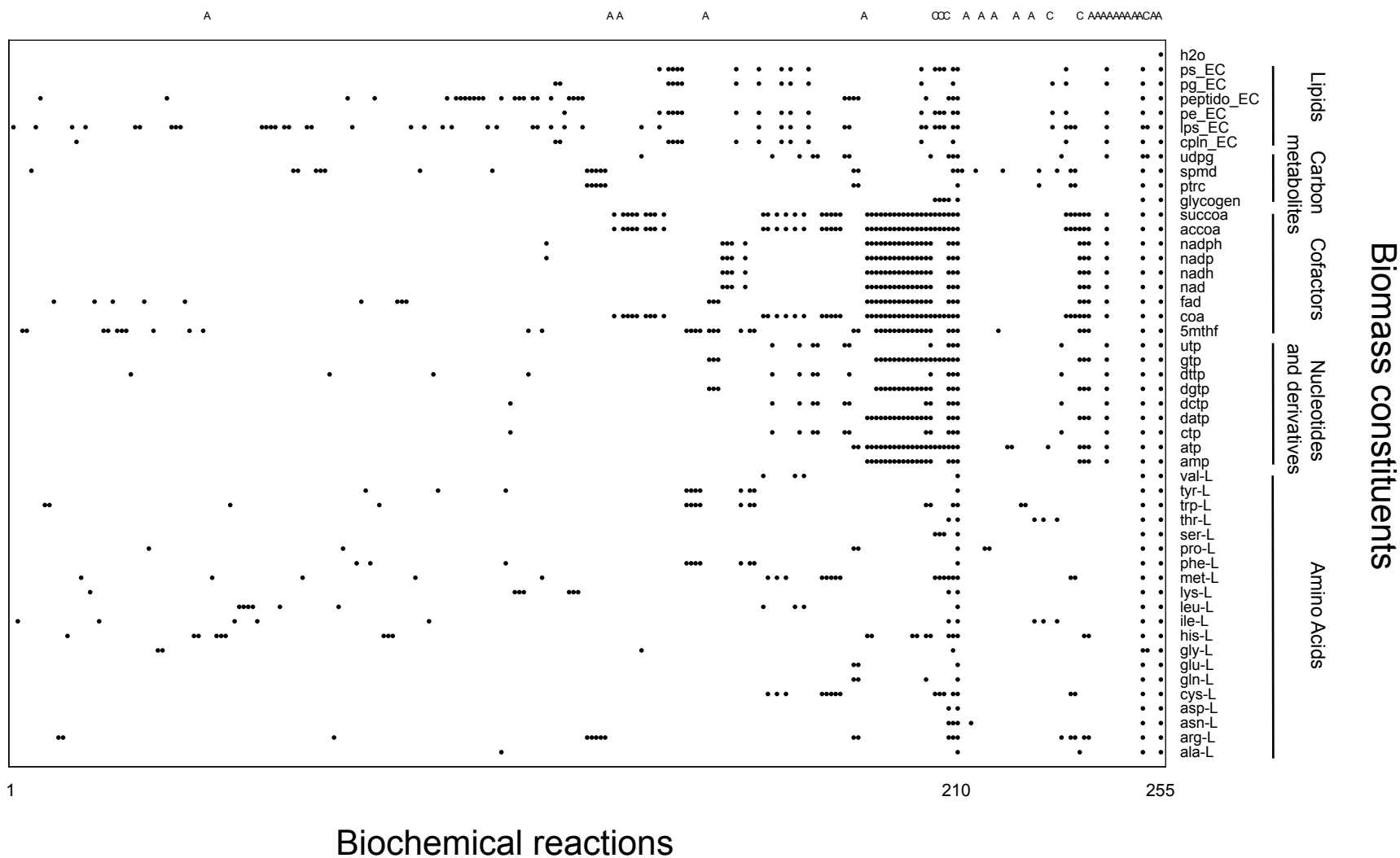


Fig. S11. Pairwise epistasis and functional association among 255 important reactions in *E. coli*. Both rows and columns represent reactions. Epistasis ϵ between reactions is shown in the lower-left triangle by the heat map. Functional association between reactions is presented in the upper-right triangle, where a grey dot is shown when two reactions have overlapping functions. Both the epistasis and reaction function is determined in the glucose minimal medium. The reactions are numbered as in Table S6.

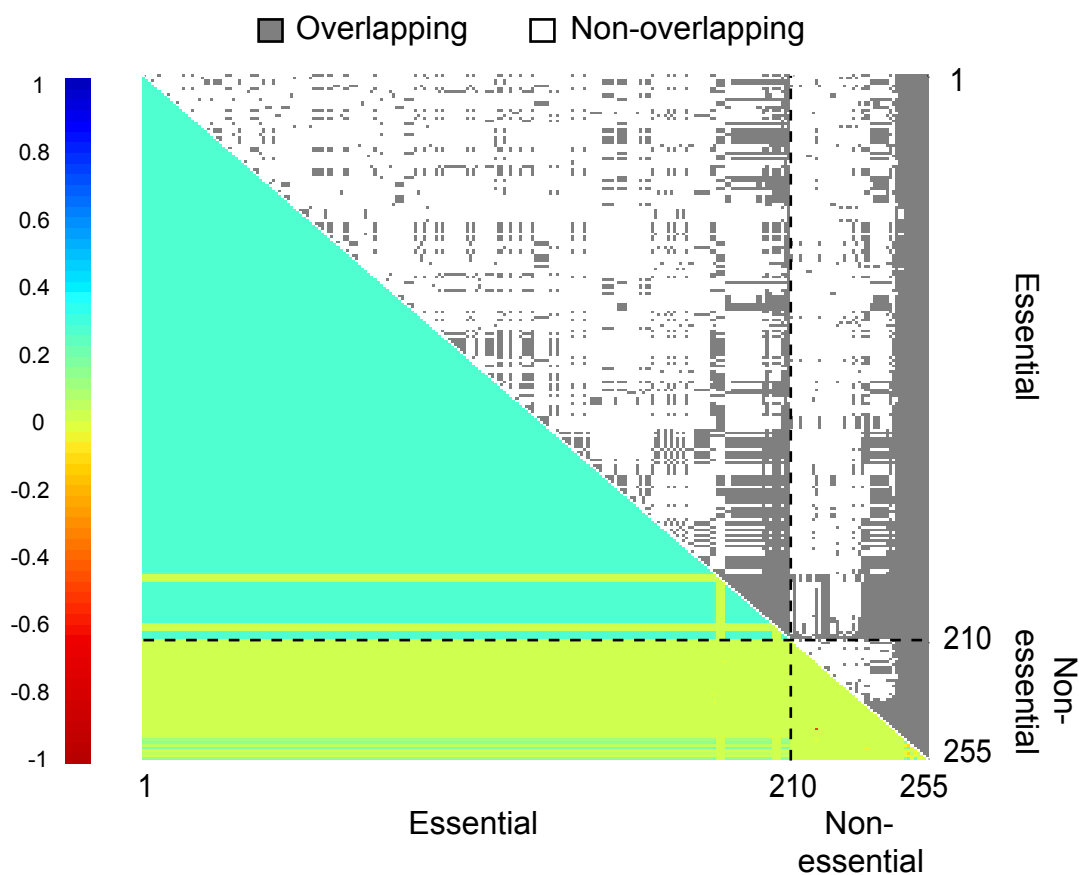


Fig. S12. Pairwise epistasis and functional association among 260 important reactions of *E. coli* under the acetate minimal medium. Both rows and columns represent reactions. Scaled epistasis between reactions is shown in the lower-left triangle by the heat map. Functional association between reactions is presented in the upper-right triangle, where a grey dot is shown when two reactions have overlapping functions. Both the epistasis and reaction function is determined in the acetate minimal medium.

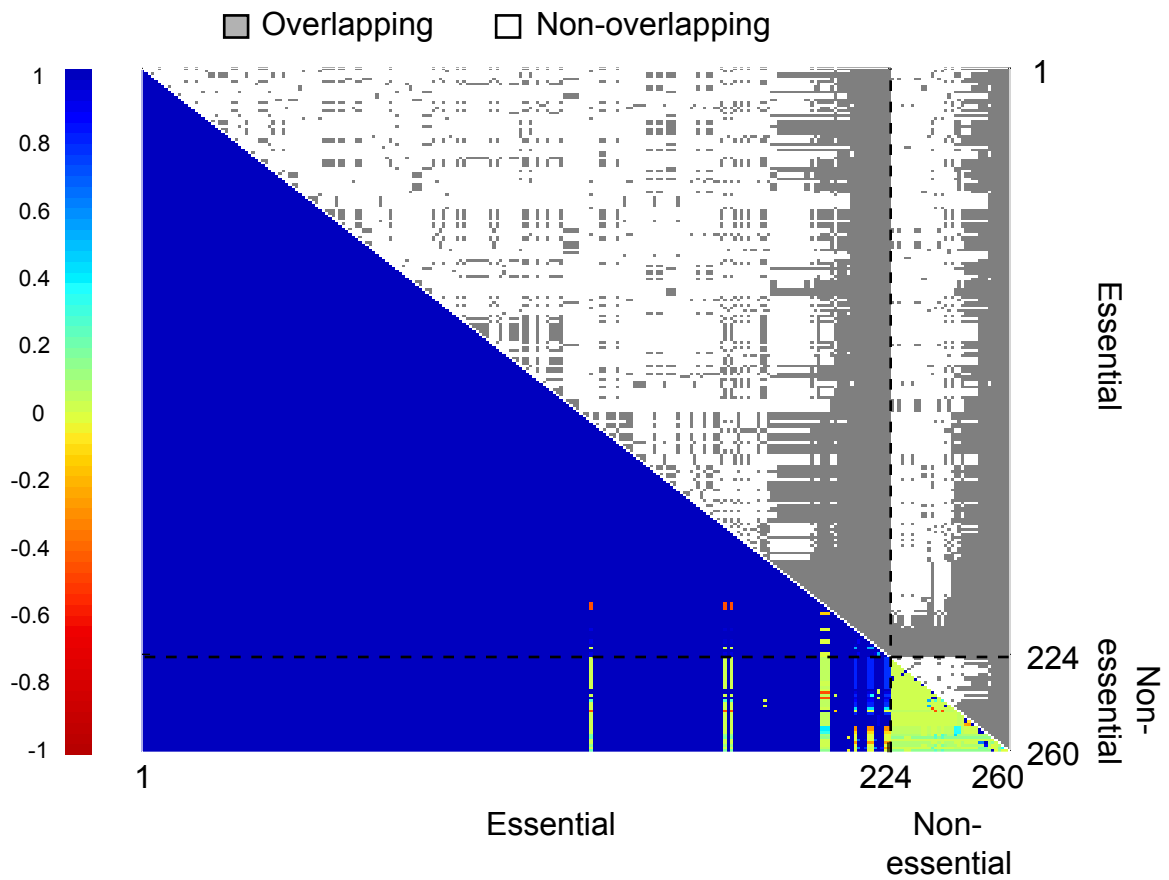


Fig. S13. Pairwise epistasis and functional association among 237 important reactions of *E. coli* under the glycerol minimal medium. Both rows and columns represent reactions. Scaled epistasis between reactions is shown in the lower-left triangle by the heat map. Functional association between reactions is presented in the upper-right triangle, where a grey dot is shown when two reactions have overlapping functions. Both the epistasis and reaction function is determined in the glycerol minimal medium.

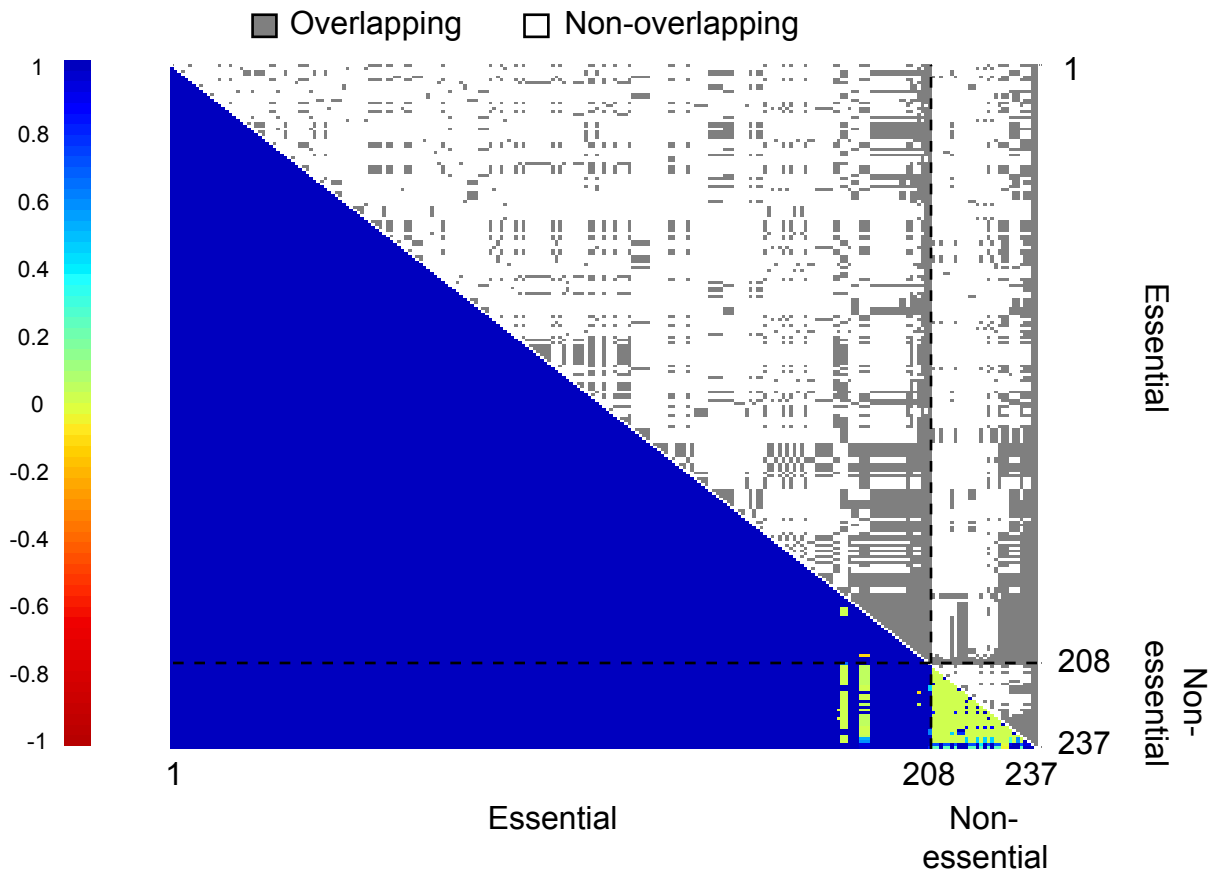


Table S6. The 255 important *E. coli* metabolic reactions with identified functions under the glucose minimal medium

Reaction number	Reaction name	Reaction formula	Biomass constituents contributed by each reaction	Functional annotation by Palsson's group ¹
1	A5PISO	ru5p_D_c <-> ara5p_c	lps_EC	Alternate Carbon Metabolism
2	ACHBS	2obut_c + h_c + pyr_c -> 2ahbut_c + co2_c	ile-L	Valine, leucine, and isoleucine metabolism
3	ADCL	4adcho_c -> 4abz_c + h_c + pyr_c	5mthf	Cofactor and Prosthetic Group Biosynthesis
4	ADCS	chor_c + gln_L_c -> 4adcho_c + glu_L_c	5mthf	Cofactor and Prosthetic Group Biosynthesis
5	ADMDCr	amet_c + h_c <-> ametam_c + co2_c	spmd	Arginine and Proline Metabolism
6	AGMHE	adphep_D_D_c -> adphep_L_D_c	lps_EC	Cell Envelope Biosynthesis
7	ALAALAR	2 ala_D_c + atp_c <-> adp_c + alaala_c + h_c + pi_c	peptido_EC	Cell Envelope Biosynthesis
8	ANPRT	anth_c + prpp_c -> ppi_c + pran_c	trp-L	Tyrosine, Tryptophan, and Phenylalanine Metabolism
9	ANS	chor_c + gln_L_c -> anth_c + glu_L_c + h_c + pyr_c	trp-L	Tyrosine, Tryptophan, and Phenylalanine Metabolism
10	APRAUR	5apru_c + h_c + nadph_c -> 5aprbu_c + nadp_c	fad	Cofactor and Prosthetic Group Biosynthesis
11	ARGSL	argsuc_c <-> arg_L_c + fum_c	arg-L	Arginine and Proline Metabolism
12	ARGSS	asp_L_c + atp_c + citr_L_c -> amp_c + argsuc_c + h_c + ppi_c	arg-L	Arginine and Proline Metabolism
13	ATPPRT	atp_c + prpp_c -> ppi_c + prbatp_c	his-L	Histidine Metabolism
14	C120SN	actACP_c + 14 h_c + 4 malACP_c + 10 nadph_c -> 4 ACP_c + 4 co2_c + ddcaACP_c + 5 h2o_c + 10 nadp_c	lps_EC	Membrane Lipid Metabolism
15	CLPNS_EC	0.04 pg_EC_c <-> 0.02 clpn_EC_c + glyc_c	clpn_EC	Membrane Lipid Metabolism
16	CYSTL	cyst_L_c + h2o_c -> hcys_L_c + nh4_c + pyr_c	met-L	Methionine Metabolism
17	DAGK_EC	0.02 12dgr_EC_c + atp_c -> adp_c + h_c + 0.02 pa_EC_c	lps_EC	Cell Envelope Biosynthesis
18	DAPDC	26dap_c + h_c -> co2_c + lys_L_c	lys-L	Threonine and Lysine Metabolism
19	DB4PS	ru5p_D_c -> db4p_c + for_c + h_c	fad	Cofactor and Prosthetic Group Biosynthesis
20	DHAD2	23dhmp_c -> 3mop_c + h2o_c	ile-L	Valine, leucine, and isoleucine metabolism
21	DHFS	atp_c + dhpt_c + glu_L_c -> adp_c + dhf_c + pi_c	5mthf	Cofactor and Prosthetic Group Biosynthesis
22	DHNPA2	dhnpt_c -> 6hnhpt_c + gcald_c	5mthf	Cofactor and Prosthetic Group Biosynthesis
23	DHPPDA2	25drapp_c + h_c + h2o_c -> 5apru_c + nh4_c	fad	Cofactor and Prosthetic Group Biosynthesis
24	DHPS2	4abz_c + 6hnhptpp_c -> dhpt_c + h_c + ppi_c	5mthf	Cofactor and Prosthetic Group Biosynthesis
25	DNMPPA	dhpmp_c + h2o_c -> dhnpt_c + pi_c	5mthf	Cofactor and Prosthetic Group Biosynthesis
26	DNTPPA	ahdt_c + h2o_c -> dhpmp_c + h_c + ppi_c	5mthf	Cofactor and Prosthetic Group Biosynthesis
27	DTMPK	atp_c + dtmp_c <-> adp_c + dtdp_c	dttp	Nucleotide Salvage Pathways

28	EDTXS1	ddcaACP_c + kdo2lipid4_c -> ACP_c + kdo2lipid4L_c	lps_EC	Cell Envelope Biosynthesis
29	EDTXS2	kdo2lipid4L_c + myrsACP_c -> ACP_c + lipa_c	lps_EC	Cell Envelope Biosynthesis
30	FMNAT	atp_c + fmn_c + h_c -> fad_c + ppi_c	fad	Cofactor and Prosthetic Group Biosynthesis
31	G5SADs	glu5sa_c -> 1pyr5c_c + h_c + h2o_c	pro-L	Arginine and Proline Metabolism
32	GCALDD	gcald_c + h2o_c + nad_c -> glyclt_c + 2 h_c + nadh_c	5mthf	Folate Metabolism
33	GLCS1	adpglc_c -> adp_c + glycogen_c + h_c	glycogen	Glycolysis/Gluconeogenesis
34	GLGC	atp_c + g1p_c + h_c -> adpglc_c + ppi_c	glycogen	Glycolysis/Gluconeogenesis
35	GLUR	glu_D_c <-> glu_L_c	peptido_EC	Cell Envelope Biosynthesis
36	GMHEPAT	atp_c + gmhep1p_c + h_c -> adphep_D_D_c + ppi_c	lps_EC	Cell Envelope Biosynthesis
37	GMHEPK	atp_c + gmhep7p_c -> adp_c + gmhep17bp_c + h_c	lps_EC	Cell Envelope Biosynthesis
38	GMHEPPA	gmhep17bp_c + h2o_c -> gmhep1p_c + pi_c	lps_EC	Cell Envelope Biosynthesis
39	GTPCII2	gtp_c + 3 h2o_c -> 25drapp_c + for_c + 2 h_c + ppi_c	fad	Cofactor and Prosthetic Group Biosynthesis
40	GTPCI	gtp_c + h2o_c -> ahdt_c + for_c	5mthf	Cofactor and Prosthetic Group Biosynthesis
41	HISTD	h2o_c + histd_c + 2 nad_c -> 3 h_c + his_L_c + 2 nadh_c	his-L	Histidine Metabolism
42	HISTP	h2o_c + hisp_c -> histd_c + pi_c	his-L	Histidine Metabolism
43	HPPK2	6hmhpt_c + atp_c -> 6hmhptpp_c + amp_c + h_c	5mthf	Cofactor and Prosthetic Group Biosynthesis
44	HSDy	hom_L_c + nadp_c <-> aspsa_c + h_c + nadph_c	met-L	Threonine and Lysine Metabolism
45	HSST	hom_L_c + succoa_c -> coa_c + suchms_c	met-L	Methionine Metabolism
46	HSTPT	glu_L_c + imacp_c -> akg_c + hisp_c	his-L	Histidine Metabolism
47	IG3PS	gln_L_c + prlp_c -> aicar_c + eig3p_c + glu_L_c + h_c	his-L	Histidine Metabolism
48	IGPDH	eig3p_c -> h2o_c + imacp_c	his-L	Histidine Metabolism
49	IGPS	2cpr5p_c + h_c -> 3ig3p_c + co2_c + h2o_c	trp-L	Tyrosine, Tryptophan, and Phenylalanine Metabolism
50	ILETA	akg_c + ile_L_c <-> 3mop_c + glu_L_c	ile-L	Valine, leucine, and isoleucine metabolism
51	IPMD	3c2hmp_c + nad_c -> 3c4mop_c + h_c + nadh_c	leu-L	Valine, leucine, and isoleucine metabolism
52	IPPMIa	3c2hmp_c <-> 2ippm_c + h2o_c	leu-L	Valine, leucine, and isoleucine metabolism
53	IPPMIb	2ippm_c + h2o_c <-> 3c3hmp_c	leu-L	Valine, leucine, and isoleucine metabolism
54	IPPS	3mob_c + accoa_c + h2o_c -> 3c3hmp_c + coa_c + h_c	leu-L	Valine, leucine, and isoleucine metabolism
55	KARA2i	2ahbut_c + h_c + nadph_c -> 23dhmp_c + nadp_c	ile-L	Valine, leucine, and isoleucine metabolism

56	KAS16	ddcaACP_c + 2 h_c + malACP_c + nadph_c -> 3hmrsACP_c + ACP_c + co2_c + nadp_c	lps_EC	Membrane Lipid Metabolism
57	KDOCT2	ctp_c + kdo_c -> ckdo_c + ppi_c	lps_EC	Cell Envelope Biosynthesis
58	KDOPP	h2o_c + kdo8p_c -> kdo_c + pi_c	lps_EC	Cell Envelope Biosynthesis
59	KDOPS	ara5p_c + h2o_c + pep_c -> kdo8p_c + pi_c	lps_EC	Cell Envelope Biosynthesis
60	LEUTAi	4mop_c + glu_L_c -> akg_c + leu_L_c	leu-L	Valine, leucine, and isoleucine metabolism
61	LPADSS	lipidX_c + u23ga_c -> h_c + lipidAds_c + udp_c	lps_EC	Cell Envelope Biosynthesis
62	LPSSYN_EC	3 adp hep_L_D_c + 2 cdpea_c + 3 ckdo_c + lipa_c + 2 udpg_c -> 3 adp_c + 2 cdp_c + 3 cmp_c + 10 h_c + lps_EC_c + 2 udp_c	lps_EC	Cell Envelope Biosynthesis
63	MDRPD	5mdru1p_c -> dkmpp_c + h2o_c	spmd	Arginine and Proline Metabolism
64	METAT	atp_c + h2o_c + met_L_c -> amet_c + pi_c + ppi_c	spmd	Methionine Metabolism
65	METS	5mthf_c + hcys_L_c -> met_L_c + thf_c	met-L	Methionine Metabolism
66	MOAT	ckdo_c + lipidA_c -> cmp_c + h_c + kdolipid4_c	lps_EC	Cell Envelope Biosynthesis
67	MOAT2	ckdo_c + kdolipid4_c -> cmp_c + h_c + kdo2lipid4_c	lps_EC	Cell Envelope Biosynthesis
68	MTAN	5mta_c + h2o_c -> 5mtr_c + ade_c	spmd	Arginine and Proline Metabolism
69	MTRI	5mdr1p_c <-> 5mdru1p_c	spmd	Arginine and Proline Metabolism
70	MTRK	5mtr_c + atp_c -> 5mdr1p_c + adp_c + h_c	spmd	Arginine and Proline Metabolism
71	NDPK4	atp_c + dtdp_c <-> adp_c + dttp_c	dttp	Nucleotide Salvage Pathways
72	OCBT	cbp_c + orn_c <-> citr_L_c + h_c + pi_c	arg-L	Arginine and Proline Metabolism
73	OMCDC	3c4mop_c + h_c -> 4mop_c + co2_c	leu-L	Valine, leucine, and isoleucine metabolism
74	P5CR	1pyr5c_c + 2 h_c + nadph_c -> nadp_c + pro_L_c	pro-L	Arginine and Proline Metabolism
75	PAPPT3	udepp_c + ugmda_c -> uagmda_c + ump_c	peptido_EC	Cell Envelope Biosynthesis
76	PEPT_EC	cmp_c + h_c + 0.02 pe_EC_c <-> 0.02 l2dgr_EC_c + cdpea_c	lps_EC	Cell Envelope Biosynthesis
77	PHETA1	akg_c + phe_L_c <-> glu_L_c + phpyr_c	phe-L	Tyrosine, Tryptophan, and Phenylalanine Metabolism
78	PMDPHT	5aprbu_c + h2o_c -> 4r5au_c + pi_c	fad	Cofactor and Prosthetic Group Biosynthesis
79	PPND	nad_c + pphn_c -> 34hpp_c + co2_c + nadh_c	tyr-L	Tyrosine, Tryptophan, and Phenylalanine Metabolism
80	PPNDH	h_c + pphn_c -> co2_c + h2o_c + phpyr_c	phe-L	Tyrosine, Tryptophan, and Phenylalanine Metabolism
81	PPTGS	uaagmda_c -> h_c + peptido_EC_c + udcpdp_c	peptido_EC	Cell Envelope Biosynthesis
82	PRAIi	pran_c -> 2cpr5p_c	trp-L	Tyrosine, Tryptophan, and Phenylalanine Metabolism
83	PRAMPC	h2o_c + prbamp_c -> prfp_c	his-L	Histidine Metabolism

84	PRATPP	h2o_c + prbatp_c -> h_c + ppi_c + prbamp_c	his-L	Histidine Metabolism
85	PRMICli	prfp_c -> prlp_c	his-L	Histidine Metabolism
86	RBFK	atp_c + ribflv_c -> adp_c + fmn_c + h_c	fad	Cofactor and Prosthetic Group Biosynthesis
87	RBFSa	4r5au_c + db4p_c -> dmlz_c + 2 h2o_c + pi_c	fad	Cofactor and Prosthetic Group Biosynthesis
88	RBFSb	2 dmlz_c -> 4r5au_c + ribflv_c	fad	Cofactor and Prosthetic Group Biosynthesis
89	S7PI	s7p_c -> gmhep7p_c	lps_EC	Cell Envelope Biosynthesis
90	SHSL1	cys_L_c + suchms_c -> cyst_L_c + h_c + succ_c	met-L	Methionine Metabolism
91	SPMS	ametam_c + ptrc_c -> 5mta_c + h_c + spmd_c	spmd	Arginine and Proline Metabolism
92	TDSK	atp_c + lipidAds_c -> adp_c + h_c + lipidA_c	lps_EC	Cell Envelope Biosynthesis
93	THRD_L	thr_L_c -> 2obut_c + nh4_c	ile-L	Valine, leucine, and isoleucine metabolism
94	TMDS	dump_c + mlthf_c -> dhf_c + dtmp_c	dttp	Nucleotide Salvage Pathways
95	TYRTA	akg_c + tyr_L_c <-> 34hpp_c + glu_L_c	tyr-L	Tyrosine, Tryptophan, and Phenylalanine Metabolism
96	U23GAAT	3hmrsACP_c + u3hga_c -> ACP_c + h_c + u23ga_c	lps_EC	Cell Envelope Biosynthesis
97	UAAGDS	26dap_c + atp_c + uamag_c -> adp_c + h_c + pi_c + ugmd_c	peptido_EC	Cell Envelope Biosynthesis
98	UAGAAT	3hmrsACP_c + uacgam_c <-> ACP_c + u3aga_c	lps_EC	Cell Envelope Biosynthesis
99	UAGCVT	pep_c + uacgam_c -> pi_c + uaccg_c	peptido_EC	Cell Envelope Biosynthesis
100	UAGPT3	uacgam_c + uagmda_c -> h_c + uaagmda_c + udp_c	peptido_EC	Cell Envelope Biosynthesis
101	UAMAGS	atp_c + glu_D_c + uama_c -> adp_c + h_c + pi_c + uamag_c	peptido_EC	Cell Envelope Biosynthesis
102	UAMAS	ala_L_c + atp_c + uamr_c -> adp_c + h_c + pi_c + uama_c	peptido_EC	Cell Envelope Biosynthesis
103	UAPGR	h_c + nadph_c + uaccg_c -> nadp_c + uamr_c	peptido_EC	Cell Envelope Biosynthesis
104	UDCPDP	h2o_c + udcpp_c -> h_c + pi_c + udcpp_c	peptido_EC	Cell Envelope Biosynthesis
105	UGMDDS	alaala_c + atp_c + ugmd_c -> adp_c + h_c + pi_c + ugmda_c	peptido_EC	Cell Envelope Biosynthesis
106	UHGADA	h2o_c + u3aga_c -> ac_c + u3hga_c	lps_EC	Cell Envelope Biosynthesis
107	UNK3	2kmb_c + glu_L_c -> akg_c + met_L_c	spmd	Arginine and Proline Metabolism
108	USHD	h2o_c + u23ga_c -> 2 h_c + lipidX_c + ump_c	lps_EC	Cell Envelope Biosynthesis
109	ALATA_L	akg_c + ala_L_c <-> glu_L_c + pyr_c	peptido_EC	Alanine and aspartate metabolism
110	CHORM	chor_c -> pphn_c	phe-L tyr-L	Tyrosine, Tryptophan, and Phenylalanine Metabolism

111	CTPS2	atp_c + gln_L_c + h2o_c + utp_c -> adp_c + ctp_c + glu_L_c + 2 h_c + pi_c	ctp dctp	Purine and Pyrimidine Biosynthesis
112	DAPE	26dap_LL_c <-> 26dap_c	lys-L peptido_EC	Threonine and Lysine Metabolism
113	DHDPRy	23dhdp_c + h_c + nadph_c -> nadp_c + thdp_c	lys-L peptido_EC	Threonine and Lysine Metabolism
114	DHDPS	aspsa_c + pyr_c -> 23dhdp_c + h_c + 2 h2o_c	lys-L peptido_EC	Threonine and Lysine Metabolism
115	DHFR	dhf_c + h_c + nadph_c <-> nadp_c + thf_c	dttp 5mthf	Cofactor and Prosthetic Group Biosynthesis
116	G1PACT	accoa_c + gam1p_c -> acgam1p_c + coa_c + h_c	lps_EC peptido_EC	Cell Envelope Biosynthesis
117	GF6PTA	f6p_c + gln_L_c -> gam6p_c + glu_L_c	lps_EC peptido_EC	Cell Envelope Biosynthesis
118	MTHFR2	h_c + mlthf_c + nadh_c -> 5mthf_c + nad_c	met-L 5mthf	Folate Metabolism
119	NADK	atp_c + nad_c -> adp_c + h_c + nadp_c	nadp nadph	Cofactor and Prosthetic Group Biosynthesis
120	PGAMT	gam1p_c <-> gam6p_c	lps_EC peptido_EC	Cell Envelope Biosynthesis
121	PGPP_EC	h2o_c + 0.02 pgp_EC_c -> 0.02 pg_EC_c + pi_c	clpn_EC pg_EC	Membrane Lipid Metabolism
122	PGSA_EC	0.02 cdpdag_EC_c + glyc3p_c <-> cmp_c + h_c + 0.02 pgp_EC_c	clpn_EC pg_EC	Membrane Lipid Metabolism
123	PSD_EC	h_c + 0.02 ps_EC_c -> co2_c + 0.02 pe_EC_c	lps_EC pe_EC	Membrane Lipid Metabolism
124	SDPDS	h2o_c + sl26da_c -> 26dap_LL_c + succ_c	lys-L peptido_EC	Threonine and Lysine Metabolism
125	SDPTA	akg_c + sl26da_c <-> glu_L_c + sl2a6o_c	lys-L peptido_EC	Threonine and Lysine Metabolism
126	THDPS	h2o_c + succoa_c + thdp_c -> coa_c + sl2a6o_c	lys-L peptido_EC	Threonine and Lysine Metabolism
127	UAGDP	acgam1p_c + h_c + utp_c -> ppi_c + uacgam_c	lps_EC peptido_EC	Cell Envelope Biosynthesis
128	ACGK	acglu_c + atp_c -> acg5p_c + adp_c	arg-L ptrc spmd	Arginine and Proline Metabolism
129	ACGS	accoa_c + glu_L_c -> acglu_c + coa_c + h_c	arg-L ptrc spmd	Arginine and Proline Metabolism
130	ACODA	acorn_c + h2o_c -> ac_c + orn_c	arg-L ptrc spmd	Arginine and Proline Metabolism
131	ACOTA	acorn_c + akg_c <-> acg5sa_c + glu_L_c	arg-L ptrc spmd	Arginine and Proline Metabolism
132	AGPR	acg5sa_c + nadp_c + pi_c <-> acg5p_c + h_c + nadph_c	arg-L ptrc spmd	Arginine and Proline Metabolism
133	ASAD	aspsa_c + nadp_c + pi_c <-> 4pasp_c + h_c + nadph_c	lys-L met-L peptido_EC	Threonine and Lysine Metabolism
134	ASP1DC	asp_L_c + h_c -> ala_B_c + co2_c	accoa coa succoa	Cofactor and Prosthetic Group Biosynthesis
135	ASPK	asp_L_c + atp_c <-> 4pasp_c + adp_c	lys-L met-L peptido_EC	Threonine and Lysine Metabolism
136	DPCOAK	atp_c + dpcoa_c -> adp_c + coa_c + h_c	accoa coa succoa	Cofactor and Prosthetic Group Biosynthesis
137	DPR	2dhp_c + h_c + nadph_c -> nadp_c + pant_c	accoa coa succoa	Cofactor and Prosthetic Group Biosynthesis
138	MOHMT	3mob_c + h2o_c + mlthf_c -> 2dhp_c + thf_c	accoa coa succoa	Cofactor and Prosthetic Group Biosynthesis
139	PANTS	ala_B_c + atp_c + pant_c -> amp_c + h_c + pnto_c + ppi_c	accoa coa succoa	Cofactor and Prosthetic Group Biosynthesis

140	PGMT	g1p_c <-> g6p_c	glycogen lps_EC udpg	Alternate Carbon Metabolism
141	PNTK	atp_c + pnto_c -> 4ppan_c + adp_c + h_c	accoa coa succoa	Cofactor and Prosthetic Group Biosynthesis
142	PPCDC	4ppcys_c + h_c -> co2_c + pan4p_c	accoa coa succoa	Cofactor and Prosthetic Group Biosynthesis
143	PPNCL2	4ppan_c + ctp_c + cys_L_c -> 4ppcys_c + cmp_c + h_c + ppi_c	accoa coa succoa	Cofactor and Prosthetic Group Biosynthesis
144	PSSA_EC	0.02 cdpdag_EC_c + ser_L_c <-> cmp_c + h_c + 0.02 ps_EC_c	lps_EC pe_EC ps_EC	Membrane Lipid Metabolism
145	PTPATi	atp_c + h_c + pan4p_c -> dpcoa_c + ppi_c	accoa coa succoa	Cofactor and Prosthetic Group Biosynthesis
146	C141SN	actACP_c + 16 h_c + 5 malACP_c + 11 nadph_c -> 5 ACP_c + 5 co2_c + 6 h2o_c + 11 nadp_c + tdeACP_c	clpn_EC pe_EC pg_EC ps_EC	Membrane Lipid Metabolism
147	C160SN	actACP_c + 20 h_c + 6 malACP_c + 14 nadph_c -> 6 ACP_c + 6 co2_c + 7 h2o_c + 14 nadp_c + palmACP_c	clpn_EC pe_EC pg_EC ps_EC	Membrane Lipid Metabolism
148	C161SN	actACP_c + 19 h_c + 6 malACP_c + 13 nadph_c -> 6 ACP_c + 6 co2_c + 7 h2o_c + hdeACP_c + 13 nadp_c	clpn_EC pe_EC pg_EC ps_EC	Membrane Lipid Metabolism
149	C181SN	actACP_c + 22 h_c + 7 malACP_c + 15 nadph_c -> 7 ACP_c + 7 co2_c + 8 h2o_c + 15 nadp_c + octeACP_c	clpn_EC pe_EC pg_EC ps_EC	Membrane Lipid Metabolism
150	CHORS	3psme_c -> chor_c + pi_c	phe-L trp-L tyr-L 5mthf	Tyrosine, Tryptophan, and Phenylalanine Metabolism
151	DDPA	e4p_c + h2o_c + pep_c -> 2dda7p_c + pi_c	phe-L trp-L tyr-L 5mthf	Tyrosine, Tryptophan, and Phenylalanine Metabolism
152	DHQD	3dhq_c <-> 3dhsk_c + h2o_c	phe-L trp-L tyr-L 5mthf	Tyrosine, Tryptophan, and Phenylalanine Metabolism
153	DHQS	2dda7p_c -> 3dhq_c + pi_c	phe-L trp-L tyr-L 5mthf	Tyrosine, Tryptophan, and Phenylalanine Metabolism
154	G3PD2	glyc3p_c + nadp_c <-> dhap_c + h_c + nadph_c	clpn_EC pe_EC pg_EC ps_EC	Alternate Carbon Metabolism
155	GK1	atp_c + gmp_c <-> adp_c + gdp_c	dgtp gtp 5mthf fad	Nucleotide Salvage Pathways
156	GMPS2	atp_c + gln_L_c + h2o_c + xmp_c -> amp_c + glu_L_c + gmp_c + 2 h_c + ppi_c	dgtp gtp 5mthf fad	Purine and Pyrimidine Biosynthesis
157	IMPD	h2o_c + imp_c + nad_c -> h_c + nadh_c + xmp_c	dgtp gtp 5mthf fad	Purine and Pyrimidine Biosynthesis
158	NADS1	atp_c + dnad_c + nh4_c -> amp_c + h_c + nad_c + ppi_c	nad nadh nadp nadph	Cofactor and Prosthetic Group Biosynthesis
159	NNAT	atp_c + h_c + nicrnt_c -> dnad_c + ppi_c	nad nadh nadp nadph	Cofactor and Prosthetic Group Biosynthesis
160	NNDPR	2 h_c + prpp_c + quln_c -> co2_c + nicrnt_c + ppi_c	nad nadh nadp nadph	Cofactor and Prosthetic Group Biosynthesis
161	PASYN_EC	glyc3p_c + 0.14 hdeACP_c + 0.04 myrsACP_c + octeACP_c + 0.72 palmACP_c + 0.1 tdeACP_c -> 2 ACP_c + 0.02 pa_EC_c	clpn_EC pe_EC pg_EC ps_EC	Membrane Lipid Metabolism

162	PSCVT	pep_c + skm5p_c <-> 3psme_c + pi_c	phe-L trp-L tyr-L 5mthf	Tyrosine, Tryptophan, and Phenylalanine Metabolism
163	QULNS	dhap_c + iasp_c -> h_c + 2 h2o_c + pi_c + quln_c	nad nadh nadp nadph	Cofactor and Prosthetic Group Biosynthesis
164	SHK3Dr	3dhsk_c + h_c + nadph_c <-> nadp_c + skm_c	phe-L trp-L tyr-L 5mthf	Tyrosine, Tryptophan, and Phenylalanine Metabolism
165	SHKK	atp_c + skm_c -> adp_c + h_c + skm5p_c	phe-L trp-L tyr-L 5mthf	Tyrosine, Tryptophan, and Phenylalanine Metabolism
166	ACCOACr	accoa_c + atp_c + hco3_c <-> adp_c + h_c + malcoa_c + pi_c	clpn_EC lps_EC pe_EC pg_EC ps_EC	Membrane Lipid Metabolism
167	ACLS	h_c + 2 pyr_c -> alac_S_c + co2_c	leu-L val-L accoa coa succoa	Valine, leucine, and isoleucine metabolism
168	ADSK	aps_c + atp_c -> adp_c + h_c + paps_c	cys-L met-L accoa coa succoa	Cysteine Metabolism
169	ASPCT	asp_L_c + cbp_c -> cbasp_c + h_c + pi_c	ctp dctp dttp utp udpg	Purine and Pyrimidine Biosynthesis
170	BPNT	h2o_c + pap_c -> amp_c + pi_c	cys-L met-L accoa coa succoa	Cysteine Metabolism
171	C140SN	actACP_c + 17 h_c + 5 malACP_c + 12 nadph_c -> 5 ACP_c + 5 co2_c + 6 h2o_c + myrsACP_c + 12 nadp_c	clpn_EC lps_EC pe_EC pg_EC ps_EC	Membrane Lipid Metabolism
172	CYSS	acser_c + h2s_c -> ac_c + cys_L_c + h_c	cys-L met-L accoa coa succoa	Cysteine Metabolism
173	DASYN_EC	ctp_c + h_c + 0.02 pa_EC_c <-> 0.02 cdpdag_EC_c + ppi_c	clpn_EC lps_EC pe_EC pg_EC ps_EC	Membrane Lipid Metabolism
174	DHAD1	23dhmb_c -> 3mob_c + h2o_c	leu-L val-L accoa coa succoa	Valine, leucine, and isoleucine metabolism
175	DHORTS	dhor_S_c + h2o_c <-> cbasp_c + h_c	ctp dctp dttp utp udpg	Purine and Pyrimidine Biosynthesis
176	KARAl	alac_S_c + h_c + nadph_c -> 23dhmb_c + nadp_c	leu-L val-L accoa coa succoa	Valine, leucine, and isoleucine metabolism
177	MCOATA	ACP_c + malcoa_c <-> coa_c + malACP_c	clpn_EC lps_EC pe_EC pg_EC ps_EC	Membrane Lipid Metabolism
178	OMPDC	h_c + orot5p_c -> co2_c + ump_c	ctp dctp dttp utp udpg	Purine and Pyrimidine Biosynthesis
179	ORPT	orot5p_c + ppi_c <-> orot_c + prpp_c	ctp dctp dttp utp udpg	Purine and Pyrimidine Biosynthesis
180	PAPSR	paps_c + trdrd_c -> 2 h_c + pap_c + so3_c + trdox_c	cys-L met-L accoa coa succoa	Cysteine Metabolism
181	SADT2	atp_c + gtp_c + h2o_c + so4_c -> aps_c + gdp_c + pi_c + ppi_c	cys-L met-L accoa coa succoa	Cysteine Metabolism
182	SERAT	accoa_c + ser_L_c <-> acser_c + coa_c	cys-L met-L accoa coa succoa	Cysteine Metabolism
183	SULR	3 h2o_c + h2s_c + 3 nadp_c <-> 5 h_c + 3 nadph_c + so3_c	cys-L met-L accoa coa succoa	Cysteine Metabolism
184	SULabc	atp_c + h2o_c + so4_e -> adp_c + h_c + pi_c + so4_c	cys-L met-L accoa coa succoa	Transport, Extracellular

185	NDPK2	atp_c + udp_c <-> adp_c + utp_c	ctp dctp utp lps_EC peptido EC udpg	Nucleotide Salvage Pathways
186	UMPK	atp_c + ump_c <-> adp_c + udp_c	ctp dctp dttp utp lps_EC peptido EC udpg	Nucleotide Salvage Pathways
187	ACONT	cit_c <-> icit_c	arg-L gln-L glu-L pro-L atp 5mthf peptido_EC ptrc spmd	Citrate Cycle (TCA)
188	CS	accoa_c + h2o_c + oaa_c -> cit_c + coa_c + h_c	arg-L gln-L glu-L pro-L atp 5mthf peptido_EC ptrc spmd	Citrate Cycle (TCA)
189	ICDHyr	icit_c + nadp_c <-> akg_c + co2_c + nadph_c	arg-L gln-L glu-L pro-L atp 5mthf peptido_EC ptrc spmd	Citrate Cycle (TCA)
190	ADSL1r	dcamp_c <-> amp_c + fum_c	his-L amp atp datp accoa coa fad nad nadh nadp nadph succoa	Purine and Pyrimidine Biosynthesis
191	ADSS	asp_L_c + gtp_c + imp_c -> dcamp_c + gdp_c + 2 h_c + pi_c	his-L amp atp datp accoa coa fad nad nadh nadp nadph succoa	Purine and Pyrimidine Biosynthesis
192	ADSL2r	25aics_c <-> aicar_c + fum_c	amp atp datp dgtp gtp 5mthf accoa coa fad nad nadh nadp nadph succoa	Purine and Pyrimidine Biosynthesis
193	AIRC2	air_c + atp_c + hco3_c -> 5caiz_c + adp_c + h_c + pi_c	amp atp datp dgtp gtp 5mthf accoa coa fad nad nadh nadp nadph succoa	Purine and Pyrimidine Biosynthesis
194	AIRC3	5aizc_c <-> 5caiz_c	amp atp datp dgtp gtp 5mthf accoa coa fad nad nadh nadp nadph succoa	Purine and Pyrimidine Biosynthesis
195	GLUPRT	gln_L_c + h2o_c + prpp_c -> glu_L_c + ppi_c + pram_c	amp atp datp dgtp gtp 5mthf accoa coa fad nad nadh nadp nadph succoa	Purine and Pyrimidine Biosynthesis
196	PRAGSr	atp_c + gly_c + pram_c <-> adp_c + gar_c + h_c + pi_c	amp atp datp dgtp gtp 5mthf accoa coa fad nad nadh nadp nadph succoa	Purine and Pyrimidine Biosynthesis
197	PRAIS	atp_c + fpram_c -> adp_c + air_c + 2 h_c + pi_c	amp atp datp dgtp gtp 5mthf accoa coa fad nad nadh nadp nadph succoa	Purine and Pyrimidine Biosynthesis
198	PRASCS	5aizc_c + asp_L_c + atp_c <-> 25aics_c + adp_c + h_c + pi_c	amp atp datp dgtp gtp 5mthf accoa coa fad nad nadh nadp nadph succoa	Purine and Pyrimidine Biosynthesis
199	PRFGS	atp_c + fgam_c + gln_L_c + h2o_c -> adp_c + fpram_c + glu_L_c + h_c + pi_c	amp atp datp dgtp gtp 5mthf accoa coa fad nad nadh nadp nadph succoa	Purine and Pyrimidine Biosynthesis

200	AICART	$10\text{fthf}_c + \text{aicar}_c \leftrightarrow \text{fprica}_c + \text{thf}_c$	his-L amp atp datp dgtp gtp 5mthf accoa coa fad nad nadh nadp nadph succoa	Purine and Pyrimidine Biosynthesis
201	IMPC	$\text{h2o}_c + \text{imp}_c \leftrightarrow \text{fprica}_c$	his-L amp atp datp dgtp gtp 5mthf accoa coa fad nad nadh nadp nadph succoa	Purine and Pyrimidine Biosynthesis
202	HCO3E	$\text{co2}_c + \text{h2o}_c \leftrightarrow \text{h}_c + \text{hco3}_c$	amp atp datp dgtp gtp 5mthf accoa clpn_EC coa fad lps_EC nad nadh nadp nadph pe_EC ps_EC succoa	Unassigned
203	GLNS	$\text{atp}_c + \text{glu}_L_c + \text{nh4}_c \rightarrow \text{adp}_c + \text{gln}_L_c + \text{h}_c + \text{pi}_c$	gln-L his-L trp-L amp atp ctp datp dctp dgtp gtp 5mthf accoa coa fad lps_EC nad nadh nadp nadph peptido_EC succoa	Glutamate metabolism
204	PRPPS	$\text{atp}_c + \text{r5p}_c \leftrightarrow \text{amp}_c + \text{h}_c + \text{prpp}_c$	his-L trp-L amp atp ctp datp dctp dgtp dttp gtp utp 5mthf accoa coa fad nad nadh nadp nadph succoa udpg	Histidine Metabolism
205	PGCD	$3\text{pg}_c + \text{nad}_c \rightarrow 3\text{php}_c + \text{h}_c + \text{nadh}_c$	cys-L gly-L his-L met-L ser-L amp atp datp dgtp dttp gtp 5mthf accoa coa fad lps_EC nad nadh nadp nadph pe_EC ps_EC succoa	Glycine and Serine Metabolism
206	PSERT	$3\text{php}_c + \text{glu}_L_c \rightarrow \text{akg}_c + \text{pser}_L_c$	cys-L gly-L his-L met-L ser-L amp atp datp dgtp dttp gtp 5mthf accoa coa fad lps_EC nad nadh nadp nadph pe_EC ps_EC succoa	Glycine and Serine Metabolism
207	PSP_L	$\text{h2o}_c + \text{pser}_L_c \rightarrow \text{pi}_c + \text{ser}_L_c$	cys-L gly-L his-L met-L ser-L amp atp datp dgtp dttp gtp 5mthf accoa coa fad lps_EC nad nadh nadp nadph pe_EC ps_EC succoa	Glycine and Serine Metabolism
208	ASPTA	$\text{akg}_c + \text{asp}_L_c \leftrightarrow \text{glu}_L_c + \text{oaa}_c$	arg-L asn-L asp-L gly-L his-L ile-L lys-L met-L thr-L amp atp ctp datp dctp dgtp dttp gtp utp 5mthf accoa coa fad nad nadh nadp nadph peptido_EC succoa udpg	Alanine and aspartate metabolism

209	PPA	$h2o_c + ppi_c \rightarrow h_c + 2 pi_c$	arg-L asn-L cys-L his-L met-L trp-L amp atp ctp datp dctp dgtp dttp gtp utp 5mthf accoa clpn_EC coa fad glycogen lps_EC nad nadh nadp nadph pe_EC peptido_EC pg_EC ps_EC spmd succoa udpg	Anaplerotic reactions
210	NH4t	$nh4_e \leftrightarrow nh4_c$	arg-L asn-L asp-L cys-L gln-L glu-L gly-L his-L ile-L leu-L lys-L met-L phe-L pro-L ser-L thr-L trp-L tyr-L val-L amp atp ctp datp dctp dgtp dttp gtp utp 5mthf accoa coa fad lps_EC nad nadh nadp nadph pe_EC peptido_EC ps_EC ptrc spmd succoa udpg	Transport, Extracellular
211	ADNK1	$adn_c + atp_c \rightarrow adp_c + amp_c + h_c$	spmd	Nucleotide Salvage Pathways
212	AKGDH	$akg_c + coa_c + nad_c \rightarrow co2_c + nadh_c + succoa_c$	atp	Citrate Cycle (TCA)
213	ASNS2	$asp_L_c + atp_c + nh4_c \rightarrow amp_c + asn_L_c + h_c + ppi_c$	asn-L	Alanine and aspartate metabolism
214	DKMPPD	$dkmpp_c + h2o_c + o2_c \rightarrow 2kmb_c + for_c + 2 h_c + pi_c$	spmd	Arginine and Proline Metabolism
215	FTHFD	$10fthf_c + h2o_c \rightarrow for_c + h_c + thf_c$	gly-L	Folate Metabolism
216	G5SD	$glu5p_c + h_c + nadph_c \rightarrow glu5sa_c + nadp_c + pi_c$	pro-L	Arginine and Proline Metabolism
217	GLU5K	$atp_c + glu_L_c \rightarrow adp_c + glu5p_c$	pro-L	Arginine and Proline Metabolism
218	GLYK	$atp_c + glyc_c \rightarrow adp_c + gly3p_c + h_c$	clpn_EC	Alternate Carbon Metabolism
219	MALS	$accoa_c + glx_c + h2o_c \rightarrow coa_c + h_c + mal_L_c$	5mthf	Anaplerotic reactions
220	PUNP1	$adn_c + pi_c \leftrightarrow ade_c + r1p_c$	spmd	Nucleotide Salvage Pathways
221	SUCD1i	$fad_c + succ_c \rightarrow fadh2_c + fum_c$	atp	Citrate Cycle (TCA)
222	SUCD4	$fadh2_c + q8_c \leftrightarrow fad_c + q8h2_c$	atp	Oxidative phosphorylation
223	SUCOAS	$atp_c + coa_c + succ_c \leftrightarrow adp_c + pi_c + succoa_c$	atp	Citrate Cycle (TCA)
224	TRPAS2	$h2o_c + trp_L_c \leftrightarrow indole_c + nh4_c + pyr_c$	trp-L	Tyrosine, Tryptophan, and Phenylalanine Metabolism
225	TRPS3	$3ig3p_c \rightarrow g3p_c + indole_c$	trp-L	Tyrosine, Tryptophan, and Phenylalanine Metabolism
226	URIDK2r	$atp_c + dump_c \leftrightarrow adp_c + dudp_c$	dttp	Nucleotide Salvage Pathways
227	DRPA	$2dr5p_c \rightarrow acald_c + g3p_c$	ile-L thr-L	Alternate Carbon Metabolism

228	ORNDc	$h_c + orn_c \rightarrow co2_c + ptrc_c$	ptrc spmd	Arginine and Proline Metabolism
229	PPM2	$2dr1p_c \leftrightarrow 2dr5p_c$	ile-L thr-L	Alternate Carbon Metabolism
230	THRAr	$thr_L_c \leftrightarrow acald_c + gly_c$	ile-L thr-L	Threonine and Lysine Metabolism
231	NDPK3	$atp_c + cdp_c \leftrightarrow adp_c + ctp_c$	lps_EC pe_EC pg_EC	Nucleotide Salvage Pathways
232	PPM	$r1p_c \leftrightarrow r5p_c$	ile-L thr-L spmd	Alternate Carbon Metabolism
233	CBMK	$atp_c + co2_c + nh4_c \rightarrow adp_c + cbp_c + 2h_c$	arg-L ctp dctp dttp utp udpg	Putative
234	CYTK1	$atp_c + cmp_c \leftrightarrow adp_c + cdp_c$	accoa clpn_EC coa lps_EC pe_EC pg_EC ps_EC succoa	Nucleotide Salvage Pathways
235	ACKr	$ac_c + atp_c \leftrightarrow actp_c + adp_c$	arg-L cys-L met-L accoa coa lps_EC ptrc spmd succoa	Pyruvate metabolism
236	PTAr	$accoa_c + pi_c \leftrightarrow actp_c + coa_c$	arg-L cys-L met-L accoa coa lps_EC ptrc spmd succoa	Pyruvate metabolism
237	GARFT	$10fthf_c + gar_c \leftrightarrow fgam_c + h_c + thf_c$	amp atp datp dgtp gtp 5mthf accoa coa fad nad nadh nadp nadph succoa	Purine and Pyrimidine Biosynthesis
238	FUM	$fum_c + h2o_c \leftrightarrow mal_L_c$	arg-L his-L amp atp datp dgtp gtp 5mthf accoa coa fad nad nadh nadp nadph succoa	Citrate Cycle (TCA)
239	MDH	$mal_L_c + nad_c \leftrightarrow h_c + nadh_c + oaa_c$	arg-L his-L amp atp datp dgtp gtp 5mthf accoa coa fad nad nadh nadp nadph succoa	Citrate Cycle (TCA)
240	MTHFC	$h2o_c + methf_c \leftrightarrow 10fthf_c$	gly-L his-L amp atp datp dgtp gtp 5mthf accoa coa fad nad nadh nadp nadph succoa	Folate Metabolism
241	MTHFD	$mlthf_c + nadp_c \leftrightarrow h_c + methf_c + nadph_c$	gly-L his-L amp atp datp dgtp gtp 5mthf accoa coa fad nad nadh nadp nadph succoa	Folate Metabolism
242	GHMT2	$ser_L_c + thf_c \rightarrow gly_c + h2o_c + mlthf_c$	gly-L his-L met-L amp atp datp dgtp dttp gtp 5mthf accoa coa fad nad nadh nadp nadph succoa	Glycine and Serine Metabolism
243	Pit2r	$h_e + pi_e \leftrightarrow h_c + pi_c$	amp atp ctp datp dctp dgtp dttp gtp utp accoa clpn_EC coa fad lps_EC nad nadh nadp nadph pe_EC pg_EC ps_EC succoa udng	Transport, Extracellular

244	PPC	$\text{co2_c} + \text{h2o_c} + \text{pep_c} \rightarrow \text{h_c} + \text{oaa_c} + \text{pi_c}$	<p>arg-L asn-L asp-L gln-L glu-L Anaplerotic reactions lys-L met-L pro-L ctp dctp dttp utp accoa coa nad nadh nadh nadph peptido_EC ptrc spmd succoa udbg</p>
245	GLUDy	$\text{glu_L_c} + \text{h2o_c} + \text{nadp_c} \leftrightarrow \text{akg_c} + \text{h_c} + \text{nadph_c} + \text{nh4_c}$	<p>arg-L asn-L asp-L cys-L gln-L Glutamate metabolism glu-L gly-L his-L ile-L leu-L lys-L met-L phe-L pro-L ser-L thr-L tyr-L val-L amp atp ctp datp dctp dgtp dttp gtp utp 5mthf accoa coa fad lps_EC nad nadh nadp nadph pe_EC peptido_EC ps_EC ptrc spmd succoa udbg</p>
246	FBA	$\text{fdp_c} \leftrightarrow \text{dhap_c} + \text{g3p_c}$	<p>arg-L asn-L asp-L cys-L gln-L Glycolysis/Gluconeogenesis glu-L gly-L his-L ile-L lys-L met-L phe-L pro-L ser-L thr-L trp-L tyr-L amp atp ctp datp dctp dgtp dttp gtp utp 5mthf accoa clpn_EC coa fad glycogen lps_EC nad nadh nadh nadph pe_EC peptido_EC pg_EC ps_EC ntrc spmd succoa udbg</p>
247	PFK	$\text{atp_c} + \text{f6p_c} \rightarrow \text{adp_c} + \text{fdp_c} + \text{h_c}$	<p>arg-L asn-L asp-L cys-L gln-L Glycolysis/Gluconeogenesis glu-L gly-L his-L ile-L lys-L met-L phe-L pro-L ser-L thr-L trp-L tyr-L amp atp ctp datp dctp dgtp dttp gtp utp 5mthf accoa clpn_EC coa fad glycogen lps_EC nad nadh nadh nadph pe_EC peptido_EC pg_EC ps_EC ntrc spmd succoa udbg</p>

248	CO2t	$\text{co2_e} \leftrightarrow \text{co2_c}$	<p>arg-L asn-L asp-L cys-L gln-L Transport, Extracellular glu-L gly-L his-L ile-L leu-L lys-L met-L phe-L pro-L thr-L trp-L tyr-L val-L amp atp ctp datp dctp dgtp dttp gtp utp 5mthf accoa clpn_EC coa fad glycogen lps_EC nad nadh nadp nadph pe_EC peptido_EC pg_EC ps_EC ptrc spmd succoa udpg</p>
249	ATPS4r	$\text{adp_c} + \text{pi_c} + 4 \text{h_e} \leftrightarrow \text{atp_c} + 3 \text{h_c} + \text{h2o_c}$	<p>arg-L asn-L asp-L cys-L gln-L Oxidative phosphorylation glu-L gly-L his-L ile-L leu-L lys-L met-L phe-L pro-L ser-L thr-L trp-L tyr-L val-L amp atp ctp datp dctp dgtp dttp gtp utp 5mthf accoa clpn_EC coa fad glycogen lps_EC nad nadh nadp nadph pe_EC peptido_EC pg_EC ps_EC ptrc spmd succoa udpg</p>
250	GAPD	$\text{g3p_c} + \text{nad_c} + \text{pi_c} \leftrightarrow \text{13dpg_c} + \text{h_c} + \text{nadh_c}$	<p>arg-L asn-L asp-L cys-L gln-L Glycolysis/Gluconeogenesis glu-L gly-L his-L ile-L leu-L lys-L met-L phe-L pro-L ser-L thr-L trp-L tyr-L val-L amp atp ctp datp dctp dgtp dttp gtp utp 5mthf accoa clpn_EC coa fad glycogen lps_EC nad nadh nadp nadph pe_EC peptido_EC pg_EC ps_EC ptrc spmd succoa udpg</p>
251	O2t	$\text{o2_e} \leftrightarrow \text{o2_c}$	<p>arg-L asn-L asp-L cys-L gln-L Transport, Extracellular glu-L gly-L his-L ile-L leu-L lys-L met-L phe-L pro-L ser-L thr-L trp-L tyr-L val-L amp atp ctp datp dctp dgtp dttp gtp utp 5mthf accoa clpn_EC coa fad glycogen lps_EC nad nadh nadp nadph pe_EC peptido_EC pg_EC ps_EC ntrc snm</p>

252	PGI	$g6p_c \leftrightarrow f6p_c$	<p>arg-L asn-L asp-L cys-L gln-L Glycolysis/Gluconeogenesis glu-L gly-L his-L ile-L leu-L lys-L met-L phe-L pro-L ser-L thr-L trp-L tyr-L val-L amp atp ctp datp dctp dgtg dttp gtp utp 5mthf accoa clpn_EC coa fad glycogen lps_EC nad nadh nadp nadph pe_EC peptido_EC pg_EC ps_EC ptrc spmd succoa udpg</p>
253	PGK	$3pg_c + atp_c \leftrightarrow 13dpg_c + adp_c$	<p>arg-L asn-L asp-L cys-L gln-L Glycolysis/Gluconeogenesis glu-L gly-L his-L ile-L leu-L lys-L met-L phe-L pro-L ser-L thr-L trp-L tyr-L val-L amp atp ctp datp dctp dgtg dttp gtp utp 5mthf accoa clpn_EC coa fad glycogen lps_EC nad nadh nadp nadph pe_EC peptido_EC pg_EC ps_EC ptrc spmd succoa udpg</p>
254	TPI	$dhap_c \leftrightarrow g3p_c$	<p>arg-L asn-L asp-L cys-L gln-L Glycolysis/Gluconeogenesis glu-L gly-L his-L ile-L leu-L lys-L met-L phe-L pro-L ser-L thr-L trp-L tyr-L val-L amp atp ctp datp dctp dgtg dttp gtp utp 5mthf accoa clpn_EC coa fad glycogen lps_EC nad nadh nadp nadph pe_EC peptido_EC pg_EC ps_EC</p>
255	H2Ot	$h2o_e \leftrightarrow h2o_c$	<p><small>ntre snm</small> arg-L asn-L asp-L cys-L gln-L Transport, Extracellular glu-L gly-L his-L ile-L leu-L lys-L met-L phe-L pro-L ser-L thr-L trp-L tyr-L val-L amp atp ctp datp dctp dgtg dttp gtp utp 5mthf accoa clpn_EC coa fad glycogen h2o lps_EC nad nadh nadp nadph pe_EC peptido_EC pg_EC ps_EC ptrc spmd succoa udpg</p>

¹Reed, J.L., Vo, T.D., Schilling, C.H. & Palsson, B.O. An expanded genome-scale model of Escherichia coli K-12 (iJR904 GSM/GPR). *Genome Biol* **4**, R54 (2003).

Table S7. Numbers of *E. coli* reaction pairs that show epistatic relationships in acetate minimal medium

Reaction pairs ¹	Functions	Epistasis ²		
		Negative	Zero	Positive
E-E	With overlap	27	3	7231
	Without overlap	0	0	17715
	Sum	27	3	24946
E-N	With overlap	60	39	3730
	Without overlap	6	40	4189
	Sum	66	79	7919
N-N	With overlap	11	152	219
	Without overlap	2	209	37
	Sum	13	361	256
All	With overlap	98	194	11180
	Without overlap	8	249	21941
	Sum	106	443	33121

¹ Pairwise relationships among 260 important reactions with identified functions. E, essential reaction; N, nonessential reaction.

² Scaled epistasis of ≥ 0.01 is considered positive, ≤ -0.01 is considered negative, and between -0.01 and 0.01 is considered zero.

Table S8. Numbers of *E. coli* reaction pairs that show epistatic relationships in glycerol minimal medium

Reaction pairs ¹	Functions	Epistasis ²		
		Negative	Zero	Positive
E-E	With overlap	3	6	4216
	Without overlap	0	0	17303
	Sum	3	6	21519
E-N	With overlap	3	50	1697
	Without overlap	0	21	4261
	Sum	3	71	5958
N-N	With overlap	4	81	78
	Without overlap	0	233	10
	Sum	4	314	88
All	With overlap	10	137	5991
	Without overlap	0	254	21574
	Sum	10	391	27565

¹ Pairwise relationships among 237 important reactions with identified functions. E, essential reaction; N, nonessential reaction.

² Scaled epistasis of ≥ 0.01 is considered positive, ≤ -0.01 is considered negative, and between -0.01 and 0.01 is considered zero.

Table S9. The 212 important yeast metabolic reactions with identified functions under the glucose minimal medium

Reaction number	Reaction name	Reaction formula	Biomass constituents contributed by each reaction	Functional annotation by Palsson's group ¹
1	13GS	[c] : udpg --> 13BDgln + h + udp	13BDgln(c)	Alternate Carbon Metabolism
2	2OXOADPtim	2oxoadp[m] --> 2oxoadp[c]	lys-L(c)	Transport, Mitochondrial
3	3MOBtm	3mob[c] <==> 3mob[m]	val-L(c)	Transport, Mitochondrial
4	AATA	[c] : 2oxoadp + glu-L <==> L2aadp + akgl	lys-L(c)	Threonine and Lysine Metabolism
5	ACGKm	[m] : acglu + atp --> acg5p + adp	arg-L(c)	Arginine and Proline Metabolism
6	ACHBSm	[m] : 2obut + h + pyr --> 2ahbut + co2	ile-L(c)	Valine, Leucine, and Isoleucine Metabolism
7	ACOTAim	[m] : acg5sa + glu-L --> acorn + akgl	arg-L(c)	Arginine and Proline Metabolism
8	AGPRim	[m] : acg5p + h + nadph --> acg5sa + nadp + pi	arg-L(c)	Arginine and Proline Metabolism
9	ANPRT	[c] : anth + prpp --> ppi + pran	trp-L(c)	Tyrosine, Tryptophan, and Phenylalanine Metabolism
10	ANS	[c] : chor + gln-L --> anth + glu-L + h + pyr	trp-L(c)	Glutamine Metabolism
11	ARGSL	[c] : argsuc <==> arg-L + fum	arg-L(c)	Arginine and Proline Metabolism
12	ARGSSr	[c] : asp-L + atp + citr-L <==> amp + argsuc + h + ppi	arg-L(c)	Arginine and Proline Metabolism
13	ASNS1	[c] : asp-L + atp + gln-L + h2o --> amp + asn-L + glu-L + h + ppi	asn-L(c)	Alanine and Aspartate Metabolism
14	ATPPRT	[c] : atp + prpp --> ppi + prbatp	his-L(c)	Histidine Metabolism
15	C24STRer	[r] : ergtetrol + h + nadph --> ergst + nadp	ergst(c)	Sterol Metabolism
16	DADK	[c] : atp + damp <==> adp + dadp	damp(c)	Nucleotide Salvage Pathway
17	DB4PS	[c] : ru5p-D --> db4p + for + h	ribflv(c)	Riboflavin Metabolism
18	DHAD2m	[m] : 23dhmp --> 3mop + h2o	ile-L(c)	Valine, Leucine, and Isoleucine Metabolism
19	DHFRi	[c] : dhf + h + nadph --> nadp + thf	dtmp(c)	Folate Metabolism
20	DOLPMMer	[r] : dolmanp --> dolp + h + mannan	mannan(c)	Glycoprotein Metabolism
21	DOLPMTcer	dolp[c] + gdpmann[c] --> dolmanp[r] + gdp[c]	mannan(c)	Glycoprotein Metabolism
22	DOLPt2er	dolp[c] + h[c] <==> dolp[r] + h[r]	mannan(c)	Transport, Endoplasmic Reticular
23	DROPPRy	[c] : 25dhpp + h + nadph --> 25dthpp + nadp	ribflv(c)	Riboflavin Metabolism
24	DRTPPD	[c] : 25dthpp + h + h2o --> 5aprbu + nh4	ribflv(c)	Riboflavin Metabolism
25	ERGSTter	ergst[r] <==> ergst[c]	ergst(c)	Transport, Endoplasmic Reticular
26	ERGTTETROLter	ergtetrol[c] <==> ergtetrol[r]	ergst(c)	Transport, Endoplasmic Reticular
27	G5SADr	[c] : glu5sa <==> 1pyr5c + h + h2o	pro-L(c)	Arginine and Proline Metabolism
28	GTPCII	[c] : gtp + (3) h2o --> 25dhpp + for + (2) h + ppi	ribflv(c)	Riboflavin Metabolism

29	HACNHm	[m] : b124tc + h2o <=> hicit	lys-L(c)	Threonine and Lysine Metabolism
30	HCITSm	[m] : accoa + akgl + h2o --> coa + h + hcit	lys-L(c)	Pyruvate Metabolism
31	HICITDm	[m] : hicit + nad <=> h + nadh + oxag	lys-L(c)	Threonine and Lysine Metabolism
32	HISTD	[c] : h2o + histd + (2) nad --> (3) h + his-L + (2) nadh	his-L(c)	Histidine Metabolism
33	HISTP	[c] : h2o + hisp --> histd + pi	his-L(c)	Histidine Metabolism
34	HSTPT	[c] : glu-L + imacp --> akgl + hisp	his-L(c)	Histidine Metabolism
35	IG3PS	[c] : gln-L + prlp --> aicar + eig3p + glu-L + h	his-L(c)	Histidine Metabolism
36	IGPDH	[c] : eig3p --> h2o + imacp	his-L(c)	Histidine Metabolism
37	IGPS	[c] : 2cpr5p + h --> 3ig3p + co2 + h2o	trp-L(c)	Tyrosine, Tryptophan, and Phenylalanine Metabolism
38	IPMD	[c] : 3c2hmp + nad --> 3c4mop + h + nadh	leu-L(c)	Valine, Leucine, and Isoleucine Metabolism
39	IPPMIa	[c] : 3c2hmp <=> 2ippm + h2o	leu-L(c)	Valine, Leucine, and Isoleucine Metabolism
40	IPPMIb	[c] : 2ippm + h2o <=> 3c3hmp	leu-L(c)	Valine, Leucine, and Isoleucine Metabolism
41	KARA2im	[m] : 2ahbut + h + nadph --> 23dhmp + nadp	ile-L(c)	Valine, Leucine, and Isoleucine Metabolism
42	LEUTA	[c] : akgl + leu-L <=> 4mop + glu-L	leu-L(c)	Valine, Leucine, and Isoleucine Metabolism
43	MAN1PT	[c] : gtp + h + man1p --> gdpmann + ppi	mannan(c)	Fructose and Mannose Metabolism
44	MAN6PI	[c] : man6p <=> f6p	mannan(c)	Fructose and Mannose Metabolism
45	MANNANter	mannan[c] <=> mannan[r]	mannan(c)	Transport, Endoplasmic Reticular
46	MCITDm	[m] : hicit <=> b124tc + h2o	lys-L(c)	Threonine and Lysine Metabolism
47	MFAPS_SC	[c] : amet + (0.01) ptdmeeta_SC --> ahcys + h + (0.01) ptd2meeta_SC	pc_SC(c)	Phospholipid Biosynthesis
48	MI1PP	[c] : h2o + mi1p-D --> inost + pi	ptd1ino_SC(c)	Phospholipid Biosynthesis
49	MI1PS	[c] : g6p --> mi1p-D	ptd1ino_SC(c)	Phospholipid Biosynthesis
50	OCBTi	[c] : cbp + orn --> citr-L + h + pi	arg-L(c)	Arginine and Proline Metabolism
51	OMCDC	[c] : 3c4mop + h --> 4mop + co2	leu-L(c)	Valine, Leucine, and Isoleucine Metabolism
52	ORNTACim	[m] : acorn + glu-L --> acglu + orn	arg-L(c)	Arginine and Proline Metabolism
53	ORNt3m	h[c] + orn[m] <=> h[m] + orn[c]	arg-L(c)	Transport, Mitochondrial
54	OXAGm	[m] : h + oxag <=> 2oxoadp + co2	lys-L(c)	Threonine and Lysine Metabolism
55	P5CR	[c] : 1pyr5c + (2) h + nadph --> nadp + pro-L	pro-L(c)	Arginine and Proline Metabolism
56	PETOHM_SC	[c] : amet + (0.01) pe_SC --> ahcys + h + (0.01) ptdmeeta_SC	pc_SC(c)	Phospholipid Biosynthesis
57	PHETA1	[c] : akgl + phe-L <=> glu-L + phpyr	phe-L(c)	Tyrosine, Tryptophan, and Phenylalanine Metabolism

58	PINOS_SC	[c] : (0.01) cdpdag_SC + inost --> cmp + h + (0.01) ptdlino_SC(c) ptdlino_SC		Phospholipid Biosynthesis
59	PMANM	[c] : man1p <==> man6p	mannan(c)	Fructose and Mannose Metabolism
60	PMDPHT	[c] : 5aprbu + h2o --> 4r5au + pi	ribflv(c)	Riboflavin Metabolism
61	PMETM_SC	[c] : amet + (0.01) ptd2meeta_SC --> ahcys + h + (0.01) pc_SC	pc_SC(c)	Phospholipid Biosynthesis
62	PPNDH	[c] : h + pphn --> co2 + h2o + phpyr	phe-L(c)	Tyrosine, Tryptophan, and Phenylalanine Metabolism
63	PRAIi	[c] : pran --> 2cpr5p	trp-L(c)	Tyrosine, Tryptophan, and Phenylalanine Metabolism
64	PRAMPC	[c] : h2o + prbamp --> prfp	his-L(c)	Histidine Metabolism
65	PRATPP	[c] : h2o + prbatp --> h + ppi + prbamp	his-L(c)	Histidine Metabolism
66	PRMICIi	[c] : prfp --> prlp	his-L(c)	Histidine Metabolism
67	RBFSa	[c] : 4r5au + db4p --> dmlz + (2) h2o + pi	ribflv(c)	Riboflavin Metabolism
68	RBFSb	[c] : (2) dmlz --> 4r5au + ribflv	ribflv(c)	Riboflavin Metabolism
69	SACCD1	[c] : L2aadp6sa + glu-L + h + nadph <==> h2o + nadp + saccrp-L	lys-L(c)	Threonine and Lysine Metabolism
70	SACCD2	[c] : h2o + nad + saccrp-L <==> akg + h + lys-L + nadh	lys-L(c)	Threonine and Lysine Metabolism
71	TMDS	[c] : dump + mlthf --> dhf + dtmp	dtmp(c)	Purine and Pyrimidine Biosynthesis
72	TRE6PP	[c] : h2o + tre6p --> pi + tre	tre(c)	Alternate Carbon Metabolism
73	TRE6PS	[c] : g6p + udpg --> h + tre6p + udp	tre(c)	Alternate Carbon Metabolism
74	TRPS1	[c] : 3ig3p + ser-L --> g3p + h2o + trp-L	trp-L(c)	Tyrosine, Tryptophan, and Phenylalanine Metabolism
75	VALTA	[c] : akg + val-L <==> 3mob + glu-L	val-L(c)	Valine, Leucine, and Isoleucine Metabolism
76	6PGLter	6pgl[c] <==> 6pgl[r]	ergst(c) zymst(c)	Transport, Endoplasmic Reticular
77	ACLSm	[m] : h + (2) pyr --> alac-S + co2	leu-L(c) val-L(c)	Valine, Leucine, and Isoleucine Metabolism
78	ADSK	[c] : aps + atp --> adp + h + paps	cys-L(c) met-L(c)	Cysteine Metabolism
79	AHCi	[c] : ahcys + h2o --> adn + hcys-L	ergst(c) pc_SC(c)	Methionine Metabolism
80	C14STR	[c] : 44mctr + h + nadph --> 44mzym + nadp	ergst(c) zymst(c)	Sterol Metabolism
81	C3STDH1	[c] : 4mzym_int1 + nad --> 4mzym_int2 + co2 + h + nadh	ergst(c) zymst(c)	Sterol Metabolism
82	C3STDH2	[c] : nad + zym_int1 --> co2 + h + nadh + zym_int2	ergst(c) zymst(c)	Sterol Metabolism
83	C3STKR1	[c] : 4mzym_int2 + h + nadph --> 4mzym + nadp	ergst(c) zymst(c)	Sterol Metabolism

84	C3STKR2	[c] : h + nadph + zym_int2 --> nadp + zymst	ergst(c) zymst(c)	Sterol Metabolism
85	C4STMO1	[c] : 44mzym + (3) h + (3) nadph + (3) o2 --> 4mzym_int1 + (4) h2o + (3) nadp	ergst(c) zymst(c)	Sterol Metabolism
86	C4STMO2	[c] : 4mzym + (3) h + (3) nadph + (3) o2 --> (4) h2o + (3) nadp + zym_int1	ergst(c) zymst(c)	Sterol Metabolism
87	CHORM	[c] : chor --> pphn	phe-L(c) tyr-L(c)	Tyrosine, Tryptophan, and Phenylalanine Metabolism
88	DHAD1m	[m] : 23dhmb --> 3mob + h2o	leu-L(c) val-L(c)	Valine, Leucine, and Isoleucine Metabolism
89	DMATT	[c] : dmpp + ipdp --> grdp + ppi	ergst(c) zymst(c)	Sterol Metabolism
90	DPMVD	[c] : 5dpmev + atp --> adp + co2 + ipdp + pi	ergst(c) zymst(c)	Sterol Metabolism
91	G6PDH2er	[r] : g6p + nadp --> 6pgl + h + nadph	ergst(c) zymst(c)	Pentose Phosphate Pathway
92	G6Pter	g6p[c] <=> g6p[r]	ergst(c) zymst(c)	Transport, Endoplasmic Reticular
93	GRTT	[c] : grdp + ipdp --> frdp + ppi	ergst(c) zymst(c)	Sterol Metabolism
94	H2Oter	h2o[c] <=> h2o[r]	ergst(c) zymst(c)	Transport, Endoplasmic Reticular
95	HMGCOAR	[c] : coa + mev-R + (2) nadp <=> (2) h + hmgcoa + (2) nadph	ergst(c) zymst(c)	Sterol Metabolism
96	HSK	[c] : atp + hom-L --> adp + h + phom	ile-L(c) thr-L(c)	Glycine and Serine Metabolism
97	IPDDI	[c] : ipdp <=> dmpp	ergst(c) zymst(c)	Sterol Metabolism
98	KARA1im	[m] : alac-S + h + nadph --> 23dhmb + nadp	leu-L(c) val-L(c)	Valine, Leucine, and Isoleucine Metabolism
99	LNSTLS	[c] : Ssq23epx --> lanost	ergst(c) zymst(c)	Sterol Metabolism
100	METAT	[c] : atp + h2o + met-L --> amet + pi + ppi	ergst(c) pc_SC(c)	Methionine Metabolism
101	O2ter	o2[c] <=> o2[r]	ergst(c) zymst(c)	Transport, Endoplasmic Reticular
102	PAPSR	[c] : paps + trdrd --> (2) h + pap + so3 + trdox	cys-L(c) met-L(c)	Cysteine Metabolism
103	PMEVK	[c] : 5pmev + atp --> 5dpmev + adp	ergst(c) zymst(c)	Sterol Metabolism
104	SQ23EPXter	Ssq23epx[r] <=> Ssq23epx[c]	ergst(c) zymst(c)	Transport, Endoplasmic Reticular
105	SQLer	[r] : h + nadph + o2 + sql --> Ssq23epx + h2o + nadp	ergst(c) zymst(c)	Sterol Metabolism
106	SQLS	[c] : (2) frdp + h + nadph --> nadp + (2) ppi + sql	ergst(c) zymst(c)	Sterol Metabolism
107	SQLter	sql[c] <=> sql[r]	ergst(c) zymst(c)	Transport, Endoplasmic Reticular
108	SULR	[c] : (3) h2o + h2s + (3) nadp <=> (5) h + (3) nadph + so3	cys-L(c) met-L(c)	Cysteine Metabolism
109	THRS	[c] : h2o + phom --> pi + thr-L	ile-L(c) thr-L(c)	Threonine and Lysine Metabolism
110	ADSL1r	[c] : dcamp <=> amp + fum	amp(c) damp(c) his-L(c)	Purine and Pyrimidine Biosynthesis
111	ADSS	[c] : asp-L + gtp + imp --> dcamp + gdp + (2) h + pi	amp(c) damp(c) his-L(c)	Purine and Pyrimidine Biosynthesis

112	ASADi	[c] : 4pasp + h + nadph --> aspsa + nadp + pi	ile-L(c) met-L(c) thr-L(c)	Alanine and Aspartate Metabolism
113	ASPKi	[c] : asp-L + atp --> 4pasp + adp	ile-L(c) met-L(c) thr-L(c)	Alanine and Aspartate Metabolism
114	CHORS	[c] : 3psme --> chor + pi	phe-L(c) trp-L(c) tyr-L(c)	Tyrosine, Tryptophan, and Phenylalanine Metabolism
115	DHQS	[c] : 2dda7p --> 3dhq + pi	phe-L(c) trp-L(c) tyr-L(c)	Tyrosine, Tryptophan, and Phenylalanine Metabolism
116	DHQTi	[c] : 3dhq --> 3dhsk + h2o	phe-L(c) trp-L(c) tyr-L(c)	Tyrosine, Tryptophan, and Phenylalanine Metabolism
117	GMPS2	[c] : atp + gln-L + h2o + xmp --> amp + glu-L + gmp + (2) h + ppi	dgmp(c) gmp(c) ribflv(c)	Purine and Pyrimidine Biosynthesis
118	IMPD	[c] : h2o + imp + nad --> h + nadh + xmp	dgmp(c) gmp(c) ribflv(c)	Purine and Pyrimidine Biosynthesis
119	METS	[c] : 5mthf + hcys-L --> h + met-L + thf	ergst(c) met-L(c) pc_SC(c)	Methionine Metabolism
120	MTHFR3	[c] : (2) h + mlthf + nadph --> 5mthf + nadp	ergst(c) met-L(c) pc_SC(c)	Folate Metabolism
121	PGMT	[c] : g1p <=> g6p	13BDgln(c) glycogen(c) tre(c)	Pentose Phosphate Pathway
122	PSCVTi	[c] : pep + skm5p --> 3psme + pi	phe-L(c) trp-L(c) tyr-L(c)	Tyrosine, Tryptophan, and Phenylalanine Metabolism
123	SHK3D	[c] : 3dhsk + h + nadph --> nadp + skm	phe-L(c) trp-L(c) tyr-L(c)	Tyrosine, Tryptophan, and Phenylalanine Metabolism
124	SHKK	[c] : atp + skm --> adp + h + skm5p	phe-L(c) trp-L(c) tyr-L(c)	Tyrosine, Tryptophan, and Phenylalanine Metabolism
125	SO4ti	so4[e] --> so4[c]	cys-L(c) met-L(c) so4(c)	Transport, Extracellular
126	UMPk	[c] : atp + ump <=> adp + udp	cmp(c) dcmp(c) dtmp(c)	Nucleotide Salvage Pathway
127	ASPCT	[c] : asp-L + cbp --> cbasp + h + pi	cmp(c) dcmp(c) dtmp(c) ump(c)	Purine and Pyrimidine Biosynthesis
128	DHORTS	[c] : dhor-S + h2o <=> cbasp + h	cmp(c) dcmp(c) dtmp(c) ump(c)	Purine and Pyrimidine Biosynthesis
129	NDPK2	[c] : atp + udp <=> adp + utp	13BDgln(c) cmp(c) glycogen(c) tre(c)	Nucleotide Salvage Pathway
130	OMPDC	[c] : h + orot5p --> co2 + ump	cmp(c) dcmp(c) dtmp(c) ump(c)	Purine and Pyrimidine Biosynthesis
131	ORPT	[c] : orot5p + ppi <=> orot + prpp	cmp(c) dcmp(c) dtmp(c) ump(c)	Purine and Pyrimidine Biosynthesis
132	ADSL2r	[c] : 25aics <=> aicar + fum	amp(c) damp(c) dgmp(c) gmp(c) ribflv(c)	Purine and Pyrimidine Biosynthesis
133	AIRCr	[c] : air + co2 <=> 5aizc + h	amp(c) damp(c) dgmp(c) gmp(c) ribflv(c)	Purine and Pyrimidine Biosynthesis

134	CBPS	[c] : (2) atp + gln-L + h2o + hco3 --> (2) adp + cbp + glu-L + (2) h + pi	arg-L(c) cmp(c) dcmp(c) dtmp(c) ump(c)	Arginine and Proline Metabolism
135	GARFTi	[c] : 10fthf + gar --> fgam + h + thf	amp(c) damp(c) dgmp(c) gmp(c) ribflv(c)	Purine and Pyrimidine Biosynthesis
136	GLUPRT	[c] : gln-L + h2o + prpp --> glu-L + ppi + pram	amp(c) damp(c) dgmp(c) gmp(c) ribflv(c)	Purine and Pyrimidine Biosynthesis
137	PRAGSr	[c] : atp + gly + pram <=> adp + gar + h + pi	amp(c) damp(c) dgmp(c) gmp(c) ribflv(c)	Purine and Pyrimidine Biosynthesis
138	PRAIS	[c] : atp + fpram --> adp + air + (2) h + pi	amp(c) damp(c) dgmp(c) gmp(c) ribflv(c)	Purine and Pyrimidine Biosynthesis
139	PRASCS	[c] : 5aize + asp-L + atp <=> 25aics + adp + h + pi	amp(c) damp(c) dgmp(c) gmp(c) ribflv(c)	Purine and Pyrimidine Biosynthesis
140	PRFGS	[c] : atp + fgam + gln-L + h2o --> adp + fpram + glu-L + h + pi	amp(c) damp(c) dgmp(c) gmp(c) ribflv(c)	Purine and Pyrimidine Biosynthesis
141	ACCOACr	[c] : accoa + atp + hco3 <=> adp + h + malcoa + pi	pa_SC(c) pc_SC(c) pe_SC(c) ps_SC(c) ptdlino_SC(c) triglyc_SC(c)	Fatty Acid Biosynthesis
142	AGAT_SC	[c] : (0.01) 1ag3p_SC + (0.02) dcacoa + (0.06) ddcacoa + (0.17) hdcoa + (0.09) ocdycacoa + (0.24) odecoa + (0.27) pmtcoa + (0.05) stcoa + (0.1) tdcoa --> coa + (0.01) pa_SC	pa_SC(c) pc_SC(c) pe_SC(c) ps_SC(c) ptdlino_SC(c) triglyc_SC(c)	Phospholipid Biosynthesis
143	AICART	[c] : 10fthf + aicar <=> fprica + thf	amp(c) damp(c) dgmp(c) gmp(c) his-L(c) ribflv(c)	Purine and Pyrimidine Biosynthesis
144	CSm	[m] : accoa + h2o + oaa --> cit + coa + h	arg-L(c) atp(c) gln-L(c) glu-L(c) lys-L(c) pro-L(c)	Citric Acid Cycle
145	DESAT18_2	[c] : h + nadph + o2 + odecoa --> (2) h2o + nadp + ocdycacoa	pa_SC(c) pc_SC(c) pe_SC(c) ps_SC(c) ptdlino_SC(c) triglyc_SC(c)	Fatty Acid Biosynthesis
146	FAS100COA	[c] : (3) h + malcoa + (2) nadph +occoa --> co2 + coa + dcacoa + h2o + (2) nadp	pa_SC(c) pc_SC(c) pe_SC(c) ps_SC(c) ptdlino_SC(c) triglyc_SC(c)	Fatty Acid Biosynthesis
147	FAS120COA	[c] : dcacoa + (3) h + malcoa + (2) nadph --> co2 + coa + ddcacoa + h2o + (2) nadp	pa_SC(c) pc_SC(c) pe_SC(c) ps_SC(c) ptdlino_SC(c) triglyc_SC(c)	Fatty Acid Biosynthesis
148	FAS80COA_L	[c] : accoa + (9) h + (3) malcoa + (6) nadph --> (3) co2 + (3) coa + (3) h2o + (6) nadp +occoa	pa_SC(c) pc_SC(c) pe_SC(c) ps_SC(c) ptdlino_SC(c) triglyc_SC(c)	Fatty Acid Biosynthesis

149	ICDHy	[c] : icit + nadp --> akgl + co2 + nadph	arg-L(c) atp(c) gln-L(c) glu-L(c) Citric Acid Cycle lys-L(c) pro-L(c)
150	IMPC	[c] : h2o + imp <=> fprica	amp(c) damp(c) dgmp(c) Purine and Pyrimidine Biosynthesis gmp(c) his-L(c) ribflv(c)
151	TRDR	[c] : h + nadph + trdox --> nadp + trdrd	cys-L(c) damp(c) dcmp(c) Purine and Pyrimidine Biosynthesis dgmp(c) dtmp(c) met-L(c)
152	ACS	[c] : ac + atp + coa --> accoa + amp + ppi	cys-L(c) met-L(c) pa_SC(c) Pyruvate Metabolism pc_SC(c) pe_SC(c) ps_SC(c) ptd1ino_SC(c) triglyc_SC(c)
153	PRPPS	[c] : atp + r5p <=> amp + h + prpp	amp(c) cmp(c) damp(c) dcmp(c) Histidine Metabolism dgmp(c) dtmp(c) gmp(c) his-L(c) ribflv(c) trp-L(c) ump(c)
154	GLNS	[c] : atp + glu-L + nh4 --> adp + gln-L + h + pi	amp(c) arg-L(c) asn-L(c) cmp(c) Glutamine Metabolism damp(c) dcmp(c) dgmp(c) dtmp(c) gln-L(c) gmp(c) his-L(c) ribflv(c) trp-L(c) ump(c)
155	PIt2r	h[e] + pi[e] <=> h[c] + pi[c]	amp(c) atp(c) cmp(c) damp(c) Transport, Extracellular dcmp(c) dgmp(c) dtmp(c) gmp(c) pa_SC(c) pc_SC(c) pe_SC(c) ps_SC(c) ptd1ino_SC(c) ump(c)
156	NH4t	nh4[e] <=> nh4[c]	ala-L(c) amp(c) arg-L(c) asn-L(c) Transport, Extracellular L(c) asp-L(c) cmp(c) cys-L(c) damp(c) dcmp(c) dgmp(c) dtmp(c) gln-L(c) glu-L(c) gly(c) gmp(c) his-L(c) ile-L(c) leu-L(c) lys-L(c) met-L(c) pc_SC(c) pe_SC(c) phe-L(c) pro-L(c) ps_SC(c) ribflv(c) ser-L(c) thr-L(c) trp-L(c) tyr-L(c) ump(c) val-L(c)

157	O2t	$o2[e] \rightleftharpoons o2[c]$	13BDgln(c) ala-L(c) amp(c) arg-L(c) asn-L(c) asp-L(c) atp(c) cmp(c) cys-L(c) damp(c) dcmp(c) dgmp(c) dtmp(c) ergst(c) gln-L(c) glu-L(c) gly(c) glycogen(c) gmp(c) h2o(c) his- L(c) ile-L(c) leu-L(c) lys-L(c) mannan(c) met-L(c) pa_SC(c) pc_SC(c) pe_SC(c) phe-L(c) pro-L(c) ps_SC(c) ptd1ino_SC(c) ribflv(c) ser-L(c) thr-L(c) tre(c) triglyc_SC(c) trp- L(c) tyr-L(c) ump(c) val-L(c) zymst(c)	Transport, Extracellular
158	GLCt1	$glc-D[e] \rightarrow glc-D[c]$	13BDgln(c) ala-L(c) amp(c) arg-L(c) asn-L(c) asp-L(c) atp(c) cmp(c) cys-L(c) damp(c) dcmp(c) dgmp(c) dtmp(c) ergst(c) gln-L(c) glu-L(c) gly(c) glycogen(c) gmp(c) h2o(c) his- L(c) ile-L(c) leu-L(c) lys-L(c) mannan(c) met-L(c) pa_SC(c) pc_SC(c) pe_SC(c) phe-L(c) pro-L(c) ps_SC(c) ptd1ino_SC(c) ribflv(c) ser-L(c) so4(c) thr-L(c) tre(c) triglyc_SC(c) trp-L(c) tyr-L(c) ump(c) val-L(c) zymst(c)	Transport, Extracellular
159	2OBUTtm	$2obut[c] \rightleftharpoons 2obut[m]$	ile-L(c)	Transport, Mitochondrial
160	3C3HMPtm	$3c3hmp[c] \rightleftharpoons 3c3hmp[m]$	leu-L(c)	Transport, Mitochondrial
161	AASAD2	$[c] : L2aadp + atp + h + nadh \rightarrow L2aadp6sa + amp + nad + ppi$	lys-L(c)	Threonine and Lysine Metabolism
162	AGTi	$[c] : ala-L + glx \rightarrow gly + pyr$	gly(c)	Glycine and Serine Metabolism

163	AKGDam	[m] : akg + h + lpam <=> co2 + sdhlam	atp(c)	Citric Acid Cycle
164	AKGDbm	[m] : coa + sdhlam --> dhlam + succoa	atp(c)	Citric Acid Cycle
165	ASPTAm	[m] : akg + asp-L <=> glu-L + oaa	arg-L(c)	Alanine and Aspartate Metabolism
166	ASPt2m	asp-L[c] + h[c] <=> asp-L[m] + h[m]	arg-L(c)	Transport, Mitochondrial
167	CHLSTI	[c] : amet + o2 + zymst --> ahcys + ergtetrol + h + (2) h2o	ergst(c)	Sterol Metabolism
168	CTPS1	[c] : atp + nh4 + utp --> adp + ctp + (2) h + pi	cmp(c)	Purine and Pyrimidine Biosynthesis
169	DAGPYP_SC	[c] : h2o + (0.01) pa_SC --> (0.01) 12dgr_SC + pi	triglyc_SC(c)	Phospholipid Biosynthesis
170	DCMPDA	[c] : dcmp + h + h2o <=> dump + nh4	dcmp(c)	Purine and Pyrimidine Biosynthesis
171	DGK1	[c] : atp + dgmp <=> adp + dgdp	dgmp(c)	Purine and Pyrimidine Biosynthesis
172	FUMm	[m] : fum + h2o <=> mal-L	atp(c)	Oxidative Phosphorylation
173	G5SD2	[c] : glu5p + h + nadh --> glu5sa + nad + pi	pro-L(c)	Arginine and Proline Metabolism
174	GLU5K	[c] : atp + glu-L --> adp + glu5p	pro-L(c)	Arginine and Proline Metabolism
175	ICDHym	[m] : icit + nadp --> akg + co2 + nadph	atp(c)	Citric Acid Cycle
176	ICL	[c] : icit --> glx + succ	gly(c)	Anaplerotic reactions
177	IPPSm	[m] : 3mob + accoa + h2o --> 3c3hmp + coa + h	leu-L(c)	Valine, Leucine, and Isoleucine Metabolism
178	PPND2	[c] : nadp + pphn --> 34hpp + co2 + nadph	tyr-L(c)	Tyrosine, Tryptophan, and Phenylalanine Metabolism
179	SUCOASm	[m] : atp + coa + succ <=> adp + pi + succoa	atp(c)	Citric Acid Cycle
180	THRD_L	[c] : thr-L --> 2obut + nh4	ile-L(c)	Threonine and Lysine Metabolism
181	ADNK1	[c] : adn + atp --> adp + amp + h	ergst(c) pc_SC(c)	Nucleotide Salvage Pathway
182	ALATA_L	[c] : akg + ala-L <=> glu-L + pyr	ala-L(c) gly(c)	Alanine and Aspartate Metabolism
183	BPNT	[c] : h2o + pap --> amp + pi	cys-L(c) met-L(c)	Cysteine Metabolism
184	PEtm_SC	pe_SC[c] <=> pe_SC[m]	pe_SC(c) pe_SC(c)	Transport, Mitochondrial
185	PSERDm_SC	[m] : h + (0.01) ps_SC --> co2 + (0.01) pe_SC	pc_SC(c) pe_SC(c)	Phospholipid Biosynthesis
186	PStm_SC	ps_SC[c] <=> ps_SC[m]	pc_SC(c) pe_SC(c)	Transport, Mitochondrial
187	SLFAT	[c] : adp + h + so4 <=> aps + pi	cys-L(c) met-L(c)	Cysteine Metabolism
188	URIDK2r	[c] : atp + dump <=> adp + dudp	dcmp(c) dtmp(c)	Nucleotide Salvage Pathway
189	GLUt7m	glu-L[c] --> glu-L[m]	arg-L(c) atp(c) lys-L(c)	Transport, Mitochondrial
190	HSDxi	[c] : aspsa + h + nadh --> hom-L + nad	ile-L(c) met-L(c) thr-L(c)	Glycine and Serine Metabolism
191	PSERS_SC	[c] : (0.01) cdpdag_SC + ser-L <=> cmp + h + (0.01) ps_SC	pc_SC(c) pe_SC(c) ps_SC(c)	Phospholipid Biosynthesis

192	DASYN_SC	[c] : ctp + h + (0.01) pa_SC <=> (0.01) cdpdag_SC + ppi	pc_SC(c) pe_SC(c) ps_SC(c) ptd1ino_SC(c)	Phospholipid Biosynthesis
193	CYTK1	[c] : atp + cmp <=> adp + cdp	cmp(c) pc_SC(c) pe_SC(c) ps_SC(c) ptd1ino_SC(c)	Nucleotide Salvage Pathway
194	NDPK3	[c] : atp + cdp <=> adp + ctp	cmp(c) pc_SC(c) pe_SC(c) ps_SC(c) ptd1ino_SC(c)	Nucleotide Salvage Pathway
195	ALDD2y	[c] : acald + h2o + nadp --> ac + (2) h + nadph	pa_SC(c) pc_SC(c) pe_SC(c) ps_SC(c) ptd1ino_SC(c) triglyc_SC(c)	Pyruvate Metabolism
196	FAS140COA	[c] : ddcacoa + (3) h + malcoa + (2) nadph --> co2 + coa + h2o + (2) nadp + tdcoa	pa_SC(c) pc_SC(c) pe_SC(c) ps_SC(c) ptd1ino_SC(c) triglyc_SC(c)	Fatty Acid Biosynthesis
197	G3PD1ir	[c] : dhap + h + nadh --> glyc3p + nad	pa_SC(c) pc_SC(c) pe_SC(c) ps_SC(c) ptd1ino_SC(c) triglyc_SC(c)	Glycerolipid Metabolism
198	GAT1_SC	[c] : (0.02) dcacoa + (0.06) ddcacoa + glyc3p + (0.17) hdcoa + (0.09) ocdycacoa + (0.24) odecoa + (0.27) pmtcoa + (0.05) stcoa + (0.1) tdcoa --> (0.01) 1ag3p_SC + coa	pa_SC(c) pc_SC(c) pe_SC(c) ps_SC(c) ptd1ino_SC(c) triglyc_SC(c)	Phospholipid Biosynthesis
199	MTHFC	[c] : h2o + methf <=> 10fthf + h	amp(c) damp(c) dgmp(c) gmp(c) his-L(c) ribflv(c)	Folate Metabolism
200	MTHFD	[c] : mlthf + nadp <=> methf + nadph	amp(c) damp(c) dgmp(c) gmp(c) his-L(c) ribflv(c)	Folate Metabolism
201	GHMT2r	[c] : ser-L + thf <=> gly + h2o + mlthf	amp(c) damp(c) dgmp(c) dtmp(c) ergst(c) gmp(c) his-L(c) met-L(c) pc_SC(c) ribflv(c)	Glycine and Serine Metabolism
202	PC	[c] : atp + hco3 + pyr --> adp + h + oaa + pi	arg-L(c) asn-L(c) asp-L(c) cmp(c) dcmp(c) dtmp(c) gln-L(c) glu-L(c) ile-L(c) lys-L(c) met-L(c) pro-L(c) thr-L(c) ump(c)	Anaplerotic reactions

203	PGCD	[c] : 3pg + nad --> 3php + h + nadh	amp(c) cys-L(c) damp(c) dgmp(c) dtmp(c) ergst(c) gmp(c) his-L(c) met-L(c) pc_SC(c) pe_SC(c) ps_SC(c) ribflv(c) ser-L(c) trp-L(c)	Glycine and Serine Metabolism
204	PSERT	[c] : 3php + glu-L --> akg + pser-L	amp(c) cys-L(c) damp(c) dgmp(c) dtmp(c) ergst(c) gmp(c) his-L(c) met-L(c) pc_SC(c) pe_SC(c) ps_SC(c) ribflv(c) ser-L(c) trp-L(c)	Glycine and Serine Metabolism
205	PSP_L	[c] : h2o + pser-L --> pi + ser-L	amp(c) cys-L(c) damp(c) dgmp(c) dtmp(c) ergst(c) gmp(c) his-L(c) met-L(c) pc_SC(c) pe_SC(c) ps_SC(c) ribflv(c) ser-L(c) trp-L(c)	Glycine and Serine Metabolism
206	ENO	[c] : 2pg <=> h2o + pep	13BDgln(c) ala-L(c) amp(c) arg-L(c) asn-L(c) asp-L(c) atp(c) cmp(c) cys-L(c) damp(c) dcmp(c) dgmp(c) dtmp(c) ergst(c) gln-L(c) glu-L(c) gly(c) glycogen(c) gmp(c) h2o(c) his- L(c) ile-L(c) leu-L(c) lys-L(c) mannan(c) met-L(c) pa_SC(c) pc_SC(c) pe_SC(c) phe-L(c) pro-L(c) ps_SC(c) ptdino_SC(c) ribflv(c) ser-L(c) thr-L(c) tre(c) triglyc_SC(c) trp- L(c) tyr-L(c) ump(c) val-L(c) zymst(c)	Glycolysis/Gluconeogenesis

207	GAPD	[c] : g3p + nad + pi \rightleftharpoons 13dpg + h + nadh	13BDgln(c) ala-L(c) amp(c) arg-L(c) asn-L(c) asp-L(c) atp(c) cmp(c) cys-L(c) damp(c) dcmp(c) dgmp(c) dtmp(c) ergst(c) gln-L(c) glu-L(c) gly(c) glycogen(c) gmp(c) h2o(c) his- L(c) ile-L(c) leu-L(c) lys-L(c) mannan(c) met-L(c) pa_SC(c) pc_SC(c) pe_SC(c) phe-L(c) pro-L(c) ps_SC(c) ptd1ino_SC(c) ribflv(c) ser-L(c) thr-L(c) tre(c) triglyc_SC(c) trp- L(c) tyr-L(c) ump(c) val-L(c) zymst(c)	Glycolysis/Gluconeogenesis
208	H2Ot	h2o[e] \rightleftharpoons h2o[c]	13BDgln(c) ala-L(c) amp(c) arg-L(c) asn-L(c) asp-L(c) atp(c) cmp(c) cys-L(c) damp(c) dcmp(c) dgmp(c) dtmp(c) ergst(c) gln-L(c) glu-L(c) gly(c) glycogen(c) gmp(c) h2o(c) his- L(c) ile-L(c) leu-L(c) lys-L(c) mannan(c) met-L(c) pa_SC(c) pc_SC(c) pe_SC(c) phe-L(c) pro-L(c) ps_SC(c) ptd1ino_SC(c) ribflv(c) ser-L(c) thr-L(c) tre(c) triglyc_SC(c) trp- L(c) tyr-L(c) ump(c) val-L(c) zymst(c)	Transport, Extracellular

209	PGK	[c] : 3pg + atp <=> 13dpg + adp	13BDgln(c) ala-L(c) amp(c) Glycolysis/Gluconeogenesis arg-L(c) asn-L(c) asp-L(c) atp(c) cmp(c) cys-L(c) damp(c) dcmp(c) dgmp(c) dtmp(c) ergst(c) gln-L(c) glu-L(c) gly(c) glycogen(c) gmp(c) h2o(c) his- L(c) ile-L(c) leu-L(c) lys-L(c) mannan(c) met-L(c) pa_SC(c) pc_SC(c) pe_SC(c) phe-L(c) pro-L(c) ps_SC(c) ptd1ino_SC(c) ribflv(c) ser-L(c) thr-L(c) tre(c) triglyc_SC(c) trp- L(c) tyr-L(c) ump(c) val-L(c) zymst(c)
210	PGM	[c] : 2pg <=> 3pg	13BDgln(c) ala-L(c) amp(c) Glycolysis/Gluconeogenesis arg-L(c) asn-L(c) asp-L(c) atp(c) cmp(c) cys-L(c) damp(c) dcmp(c) dgmp(c) dtmp(c) ergst(c) gln-L(c) glu-L(c) gly(c) glycogen(c) gmp(c) h2o(c) his- L(c) ile-L(c) leu-L(c) lys-L(c) mannan(c) met-L(c) pa_SC(c) pc_SC(c) pe_SC(c) phe-L(c) pro-L(c) ps_SC(c) ptd1ino_SC(c) ribflv(c) ser-L(c) thr-L(c) tre(c) triglyc_SC(c) trp- L(c) tyr-L(c) ump(c) val-L(c) zymst(c)

211	PYK	[c] : adp + h + pep --> atp + pyr	13BDgln(c) ala-L(c) amp(c) arg-L(c) asn-L(c) asp-L(c) atp(c) cmp(c) cys-L(c) damp(c) dcmp(c) dgmp(c) dtmp(c) ergst(c) gln-L(c) glu-L(c) gly(c) glycogen(c) gmp(c) h2o(c) his- L(c) ile-L(c) leu-L(c) lys-L(c) mannan(c) met-L(c) pa_SC(c) pc_SC(c) pe_SC(c) phe-L(c) pro-L(c) ps_SC(c) ptd1ino_SC(c) ribflv(c) ser-L(c) thr-L(c) tre(c) triglyc_SC(c) trp- L(c) tyr-L(c) ump(c) val-L(c) zymst(c)	Glycolysis/Gluconeogenesis
212	TPI	[c] : dhap <==> g3p	13BDgln(c) ala-L(c) amp(c) arg-L(c) asn-L(c) asp-L(c) atp(c) cmp(c) cys-L(c) damp(c) dcmp(c) dgmp(c) dtmp(c) ergst(c) gln-L(c) glu-L(c) gly(c) glycogen(c) gmp(c) h2o(c) his- L(c) ile-L(c) leu-L(c) lys-L(c) mannan(c) met-L(c) pa_SC(c) pc_SC(c) pe_SC(c) phe-L(c) pro-L(c) ps_SC(c) ptd1ino_SC(c) ribflv(c) ser-L(c) thr-L(c) tre(c) triglyc_SC(c) trp- L(c) tyr-L(c) ump(c) val-L(c) zymst(c)	Glycolysis/Gluconeogenesis

¹Mo, M.L., Palsson, B.O., Herrgard, M.J., Connecting extracellular metabolomic profiles to intracellular metabolic states in yeast. *BMC Systems Biology* 3:37 (2009)

Table S10. Numbers of *E. coli* reaction pairs that show epistatic relationships in glucose minimal medium

Reaction pairs ¹	Functions ²	Epistasis ³		
		Negative	Zero	Positive
E-E	With overlap	0	0	1981
	Without overlap	0	9	19955
	Sum	0	9	21936
E-N	With overlap	2	1	217
	Without overlap	1	116	9113
	Sum	3	117	9330
N-N	With overlap	3	18	39
	Without overlap	20	588	322
	Sum	23	606	361
All	With overlap	5	19	2237
	Without overlap	21	713	29390
	Sum	26	732	31627

¹ Pairwise relationships among 255 important reactions. E, essential reaction; N, nonessential reaction.

² Functional annotation follows Reed et al. *Genome Biol* 4, R54 (2003).

³ Scaled epistasis of ≥ 0.01 is considered positive, ≤ -0.01 is considered negative, and between -0.01 and 0.01 is considered zero.

Table S11. Numbers of *E. coli* reaction pairs that show epistatic relationships in glucose minimal medium in the analysis by minimization of metabolic adjustment (MOMA)

Reaction pairs ¹	Functions	Epistasis ²		
		Negative	Zero	Positive
E-E	With overlap	18	0	4818
	Without overlap	0	0	16074
	Sum	18	0	20892
E-N	With overlap	39	20	2771
	Without overlap	2	6	6993
	Sum	41	26	9764
N-N	With overlap	10	10	452
	Without overlap	6	10	640
	Sum	16	20	1092
All	With overlap	67	30	8041
	Without overlap	8	16	23707
	Sum	75	46	31748

¹ Pairwise relationships among 253 important reactions (two reactions are excluded in the analysis because these reactions are essential with up to 50% flux constraint under MOMA. Nine reaction pairs are further excluded in this tabulation because their double perturbation MOMA solutions cannot be obtained. E, essential reaction; N, nonessential reaction.

² Scaled epistasis of ≥ 0.01 is considered positive, ≤ -0.01 is considered negative, and between -0.01 and 0.01 is considered zero.

Table S12. Primers used in this study

	For <i>URA3</i> deletion*
URA3_YAR002W_UP	5'- ATCAAATAACAGACCTTTACATCAAATAAGCACCCGCAAGA TATCCTAAAATCGACATCCAagcttttcaattcaattcatcattt -3'
URA3_YAR002W_DOWN	5'- ACAAACTTACGTATTGAGTTGGGCTATACGGTAATTATGT CACGGCTAAAATTTTCATTAgggtaataactgatataattaaatt -3'
URA3_YDL193W_UP	5'- AAATTACACAATTTTTGATACAATATATATACATATATTTTT CCCCATTGTTTTCTTAGagcttttcaattcaattcatcattt -3'
URA3_YDL193W_DOWN	5'- CAACTGTGCAAAATTAAGGTTGAGCGAGATACTTATCT TTTCGTTTAGCTCTTAAGTAgggtaataactgatataattaaatt -3'
URA3_YFL039C_UP	5'- TTAGATTTTTACGCTTACTGCTTTTTTCTCCCAAGATCGA AAATTTACTGAATTAACAagcttttcaattcaattcatcattt -3'
URA3_YFL039C_DOWN	5'- TTTAAAATAGAAATAGAGAGAGAGGTACATACATAAACA TACGCGCACAAAAGCAGAGAgggtaataactgatataattaaatt -3'
URA3_YHR143W-A_UP	5'- ACTTATTCTACCAACATTTAATAAGAACGTACCCAAGAATA AAAGTCCACAAGTATAACAagcttttcaattcaattcatcattt -3'
URA3_YHR143W-A_DOWN	5'- AGGTTCTTTATTTTCGTATTATTCTGCCCGGTAACCCATT ATGGTTTTAGCTCTCCATgggtaataactgatataattaaatt -3'
URA3_YIL142W_UP	5'- TGAAAGGTGGCCATTAATAGGCAAGAAGAGTCAGTCGT ACAAAGCTCAAAGATTCAGGagcttttcaattcaattcatcattt -3'
URA3_YIL142W_DOWN	5'- TTCTAATTATAATAATGACAGTGTGGCGGTTTATTATTTTC GAGGTATATCCTAAATATGgggtaataactgatataattaaatt -3'
URA3_YJR123W_UP	5'- TAATTACACTCCTTTATAATACCAAGAAAAGAGACTAGAA ATAACCGACCATTCCAAAGagcttttcaattcaattcatcattt -3'
URA3_YJR123W_DOWN	5'- TAAAAGTAATAATAAGAAAAACATATGTAATATTGAAAATCT TTCACTTTTTTAGCTTCgggtaataactgatataattaaatt -3'
URA3_YKL006W_UP	5'- ATAACAAATTTAATTGTTTGTCTTAGTGAACAAAGAGAGTC GTGAAAAATAAAATAAACAagcttttcaattcaattcatcattt -3'
URA3_YKL006W_DOWN	5'- CACCAGTTAAAGATAAAATTACATACGAAATATATCAGAAT TAAAGATCATGTACATATTgggtaataactgatataattaaatt -3'
URA3_YPR181C_UP	5'- AATTAAGTAAAGCCAGTGTATCTGTCTGTCGCCTGCCT CCCCTTCTCTTTTCTAGCCagcttttcaattcaattcatcattt -3'
URA3_YPR181C_DOWN	5'- GCCTTATATTATACAAAAAGCCTAATAACGATCAAATAAAA CAAAAGAAAGCCGTTGCTCgggtaataactgatataattaaatt -3'
URA3_YBR243C_UP	5'- GGAAGACACGCTATAGGCGCTATCATCTTTGGCCACGA CAACCTATGGACAGTACGTTGAagcttttcaattcaattcatcattt -3'
URA3_YBR243C_DOWN	5'- TTATCATAAATACCAACTTTTTCGTCATAAAAGTACAAA GTAACCTACCAATACATAATCTgggtaataactgatataattaaatt -3'
URA3_YLR088W_UP	5'- TTAGCGTCATCGTTTGAATCTAAATCTGTCGTAGTAAGG TCGAAAGAAAAGCAAAGTTAGagcttttcaattcaattcatcattt -3'
URA3_YLR088W_DOWN	5'- GTATAAAATCAATTGCGTAGTTCGAACACCCGTTAATCC TTTACTTCTTACCAATGGCACgggtaataactgatataattaaatt -3'
URA3_YJL029C_UP	5'- GCTTATTGTCACCTTACTGCATTTTCCAAACATCTTCCATT TTTGTTTTCTCAGAAGGACAgcttttcaattcaattcatcattt -3'
URA3_YJL029C_DOWN	5'- TTTATGAACTATATTATACTATACTACGTACTTCTCTGT AAACCACTTCATGCGTTTTTgggtaataactgatataattaaatt -3'
URA3_YMR307W_UP	5'- TCAGTTTTTATTTTTAAAGTCTGATAAAACAAAACAACA AACACAGCTAAATCTCAACAagcttttcaattcaattcatcattt -3'
URA3_YMR307W_DOWN	5'- ACCCCACAATGAAATAAGATACCATACTTATCGAGTTA TTATGTATGTGTCGAAGCTTTgggtaataactgatataattaaatt -3'
URA3_YNL080C_UP	5'- TCCCTTCCCTTCTTTGGTCAAATAATCTTATTTGATTCT TCAGGGTTTTAAAAAACCAGagcttttcaattcaattcatcattt -3'
URA3_YNL080C_DOWN	5'- GTTAGAGCTGTTGCAGCATATACATATACATATATATTAT ATATTTTTCTTTATTTTTTgggtaataactgatataattaaatt -3'

URA3_YOL064C_UP	5'- AATTTATTAGTTAGTAAGTAAGAAGTTTAAAGACAACCTCA GAAGACATCAGCACTTTACTagcttttcaattcaattcatcattt -3'
URA3_YOL064C_DOWN	5'- CACACATATATATATGTACTCATATATTTATGTCTATCAA TAAAGTAAATATATGTTATgggtaataactgatataattaaatt -3'
URA3_YGR157W_UP	5'- ATTCCGCCCTGAATATTTTCGAGTGATTTTCTTAGTGACAA AGCTTTTTCTTCATCTGTAGagcttttcaattcaattcatcattt -3'
URA3_YGR157W_DOWN	5'- TTCAAAATGTTAACTTGAATCCTAGTACTTTTTAAATATAT ATACTCAAAAAAAAAAAAAACgggtaataactgatataattaaatt -3'
URA3_HO_UP	5'- ATGCTTTCTGAAAACACGACTATTCTGATGGCTAACGGTG AAATTAAGACATCGCAAAACagcttttcaattcaattcatcattt -3'
URA3_HO_DOWN	5'- GTTGTTACCACAACCTTATGAGGCCCGCGGACAGCATCA AACTGTAAGATTCCGCCACAgggtaataactgatataattaaatt -3'

For *LEU2* deletion*

LEU2_YAR002W_UP	5'- ATCAAATAACAGACCTTTACATCAAATAAGCACCGCAAGAT ATCCTAAAATCGACATCCAaagagttcgaatctcttagcaacca -3'
LEU2_YAR002W_DOWN	5'- ACAAACTTACGTATTGAGTTGGGCTATACGGTAATTATGTC ACGGCTAAAATTTTCATTActccatcaaatggcagggtcattga -3'
LEU2_YDL193W_UP	5'- AAATTACACAATTTTTGATACAATATATACATATATTTTC CCCATTGTTTTCTTAGaagagttcgaatctcttagcaacca -3'
LEU2_YDL193W_DOWN	5'- CAACTGTGCAAAATTAAGGTTGAGCGAGATACTTATCT TTTCGTTTAGCTCTTAAGTActccatcaaatggcagggtcattga -3'
LEU2_YFL039C_UP	5'- TTAGATTTTTACGCTTACTGCTTTTTTCTTCCAAGATCGA AAATTTACTGAATTAACAaagagttcgaatctcttagcaacca -3'
LEU2_YFL039C_DOWN	5'- TTTAAAAATAGAAATAGAGAGAGAGAGGTACATACATAAACA TACGCGCACAAGCAGAGActccatcaaatggcagggtcattga -3'
LEU2_YHR143W-A_UP	5'- ACTTATTCTACCAACATTTAATAAGAACGTACCCAAGAAT AAAAGTCCACAAGTATAACAaagagttcgaatctcttagcaacca -3'
LEU2_YHR143W-A_DOWN	5'- AGGTTCTTTATTTTCGTATTATTCTGCCCGGTAACCCATT ATGGTTTTCAGTCTCTCCATctccatcaaatggcagggtcattga -3'
LEU2_YIL142W_UP	5'- TGAAAGGTGGCCATTAATAGGCAAGAAGAGTCAGTCGTA CAAAGCTCAAAGATTCAGGaagagttcgaatctcttagcaacca -3'
LEU2_YIL142W_DOWN	5'- TTCTAATTATAAATAATGACAGTGTGGCGGTTTATTATTTTC GAGGTATATCCTAAATATGctccatcaaatggcagggtcattga -3'
LEU2_YJR123W_UP	5'- TAATTACACTCCTTTTATAATACCAAGAAAAGAGACTAGAA ATAACCGACCATTCCAAAGaagagttcgaatctcttagcaacca -3'
LEU2_YJR123W_DOWN	5'- TAAAAGTAATAATAAGAAAAACATATGTAATATTGAAAATC TTTCACTTTTTTTAGCTTctccatcaaatggcagggtcattga -3'
LEU2_YKL006W_UP	5'- ATAACAAATTTAATTGTTTGTCTTAGTGAACAAAGAGAGT CGTGAAAAATAAAATAAACaagagttcgaatctcttagcaacca -3'
LEU2_YKL006W_DOWN	5'- CACCAGTTAAAGATAAAATTACATACGAAATATATCAGAATT AAAGATCATGTACATATTctccatcaaatggcagggtcattga -3'
LEU2_YPR181C_UP	5'- AATTAAGTGAAGCCAGTGTATCTGTCTGTGCGCTGCCTC CCCTTCTCTTTTCTAGCCaagagttcgaatctcttagcaacca -3'
LEU2_YPR181C_DOWN	5'- GCCTTATATTATACAAAAAGCCTAATAACGATCAAATAAA ACAAAAGAAAAGCCGTTGCTCctccatcaaatggcagggtcattga -3'
LEU2_YBR243C_UP	5'- GGAAGACAGCTATAGGCGCTATCATCTTTGGCCACGACA ACCTATGGACAGTACGTTGAaagagttcgaatctcttagcaacca -3'
LEU2_YBR243C_DOWN	5'- TTATCATAAATACCAACTTTTGCCTCATAAAAGTACAAAG TAACTACCAATACATAATCTctccatcaaatggcagggtcattga -3'
LEU2_YDR454C_UP	5'- AATGATGATTTGGACAAGGCCTACAAGGAATTGAAGGATT TTATCTTTGCAGAAAATGAaagagttcgaatctcttagcaacca -3'
LEU2_YDR454C_DOWN	5'- CGCTTTGCGACAGCACTCTCCTACCAGGTACATACTTAGT AATGTCTATATAGGGCTACActccatcaaatggcagggtcattga -3'
LEU2_YLR088W_UP	5'- TTAGCGTCATCGTTTGAATCTAAATCTGTCTGTAGTAAGGTC GAAAGAAAAGCAAAGTTAGaagagttcgaatctcttagcaacca -3'

LEU2_YLR088W_DOWN	5'- GTATAAAATCAATTGCGTAGTTCGAACACCCGTTAATCCTT TACTTCTTACCAATGGCACctccatcaaatggcaggcattga -3'
LEU2_YOL078W_UP	5'- ACTGAGATCGTGACGCGATTACAAAACCTTATTAAGCGCTTA TAGAATGAATCATAAATAAaagagttcgaatctcttagcaacca -3'
LEU2_YOL078W_DOWN	5'- TACCATATTTAGATATAAACCAAATTAATGCTGGTAAGAT GCAGTATAATGGGTTTTACctccatcaaatggcaggcattga -3'
LEU2_YJL029C_UP	5'- GCTTATTGTCACCTTACTGCATTTTCACAACATCTCCATTTT TGTTTTCTCAGAAGGACAaagagttcgaatctcttagcaacca -3'
LEU2_YJL029C_DOWN	5'- TTTATGAACTATATTATACTATACTACGTA CTTCCTCTGTAA ACCACTTCATGCGTTTTTctccatcaaatggcaggcattga -3'
LEU2_YMR307W_UP	5'- TCAGTTTTTATTTTTAAAGTCTGATAAAACAAAACAACAAA CACAGCTAAATCTCAACAaagagttcgaatctcttagcaacca -3'
LEU2_YMR307W_DOWN	5'- ACCCCACAATGAAATAAGATACCATACCTTATCGAGTTATT ATGTATGTGTCGAAGCTTTctccatcaaatggcaggcattga -3'
LEU2_YNL080C_UP	5'- TCCCTTCCCTTCCCTTTGGTCAAATAATCTTATTTGATTCTTC AGGGTTTTAAAAACCAGaagagttcgaatctcttagcaacca -3'
LEU2_YNL080C_DOWN	5'- GTTAGAGCTGTTGCAGCATATACATATACATATATATTATAT ATTTTTCTTTATTTTTTctccatcaaatggcaggcattga -3'
LEU2_YOL064C_UP	5'- AATTTATTAGTTAGTAAGTAAGAAGTTTAAAGACA ACTCAG AAGACATCAGCACTTTACTaagagttcgaatctcttagcaacca -3'
LEU2_YOL064C_DOWN	5'- CACACATATATATATGTACTCATATATTTATGTCTATCAATA AAGTAAATATATGTTATctccatcaaatggcaggcattga -3'
LEU2_YGR157W_UP	5'- ATTCCGCCCTGAATATTTGAGTGATTTTCTTAGTGACAAA GCTTTTTCTTCATCTGTAGaagagttcgaatctcttagcaacca -3'
LEU2_YGR157W_DOWN	5'- TTCAAAATGTTAACTTGAATCCTAGTACTTTTTAAATATATA TACTCAAAAAAAAAAACctccatcaaatggcaggcattga -3'
LUE2_HO_UP	5'- ATGCTTTCTGAAAACACGACTATTCTGATGGCTAACGGTG AAATTAAGACATCGCAAACaagagttcgaatctcttagcaacca -3'
LUE2_HO_DOWN	5'- GTTGTTACCACA ACTCTTATGAGGCCCGCGGACAGCATCA AACTGTAAGATTCCGCCACActccatcaaatggcaggcattga -3'

For colony PCR confirmation

colony_YAR002W_UP	5'- CTTATTGTAATGTGTCATTTTAAAC -3'
colony_YAR002W_DOWN	5'- ATATCATCTTGGAAATGGTATTTTAC -3'
colony_YDL193W_UP	5'- TAGTATTCGCATAACATTAGTTGGC -3'
colony_YDL193W_DOWN	5'- CTTTCTTAAGTATCAGACGAGAATT -3'
colony_YFL039C_UP	5'- ATAGGATCTTCTACTACATCAGCTT -3'
colony_YFL039C_DOWN	5'- TATTTTTATTTTATTGAGAGGGTGG -3'
colony_YHR143W-A_UP	5'- AAGTGAAGAGTATTCCACATCATCG -3'
colony_YHR143W-A_DOWN	5'- ATATTGCAAAAACCTTGAAAACCT -3'
colony_YIL142W_UP	5'- AAGAGCAGAGCAATAAAAAAGAGGA -3'
colony_YIL142W_DOWN	5'- ATAGTGGATAATTTTTGCGTTCTGT -3'
colony_YJR123W_UP	5'- TTTGAATTA AAATTA CTTTTTATTT -3'
colony_YJR123W_DOWN	5'- TAAACAATAGAGTGTACAAAGAAGA -3'
colony_YKL006W_UP	5'- TAAGTTGATCAGGTCTGCCGATTGT -3'
colony_YKL006W_DOWN	5'- ATTGTAGTTTATTTATTTAAAAGAAT -3'
colony_YPR181C_UP	5'- TCTTTCCACGTT CAGCAAGACACAC -3'
colony_YPR181C_DOWN	5'- CTTGCACGCGCAATGGACTGAACAG -3'
colony_YBR243C_UP	5'- GGAAGACACGCTATAGGCGC -3'
colony_YBR243C_DOWN	5'- TTATCATAAATACCAACTTT -3'
colony_YDR454C_UP	5'- AATGATGATTTGGACAAGGC -3'
colony_YDR454C_DOWN	5'- CGCTTTGCGACAGCACTCTC -3'
colony_YLR088W_UP	5'- TTAGCGTCATCGTTTGAATC -3'

colony_YLR088W_DOWN	5'- GTATAAAATCAATTGCGTAG -3'
colony_YOL078W_UP	5'- ACTGAGATCGTGACGCGATT -3'
colony_YOL078W_DOWN	5'- TACCATATTTAGATATAAAC -3'
colony_YJL029C_UP	5'- GCTTATTGTCACCTTACTGCA -3'
colony_YJL029C_DOWN	5'- TTTATGAACTATATTATACT -3'
colony_YMR307W_UP	5'- TCAGTTTTTATTTTTAAAGT -3'
colony_YMR307W_DOWN	5'- ACCCCACAATGAAATAAGAT -3'
colony_YNL080C_UP	5'- TCCCTTCCCTTCCTTTGGTC -3'
colony_YNL080C_DOWN	5'- GTTAGAGCTGTTGCAGCATA -3'
colony_YOL064C_UP	5'- AATTTATTAGTTAGTAAGTA -3'
colony_YOL064C_DOWN	5'- CACACATATATATGTACT -3'
colony_YGR157W_UP	5'- ATTCCGCCCTGAATATTTTCG -3'
colony_YGR157W_DOWN	5'- TTCAAAATGTTAACTTGAAT -3'
colony_HO_UP	5'- ATGCTTTCTGAAAACACGAC -3'
colony_HO_DOWN	5'- GTTGTTACCACAACCTTAT -3'

For vYFP marked strain

URA_MET_R	5'- GTGGTGGTTGGCAAATGACTAATTAAGTTAGTCAAGGCGC CATCCagcttttcaattcaattcatca -3'
URA_MET_L	5'- CATTTC AATAAAAGTTCGTTTTATAAAAGTATAGTACTTGTG AGAGgggtaataactgatataattaa -3'
URA_MET_colony_R	5'- GTGGTGGTTGGCAAATGACT -3'
URA_MET_colony_L	5'- TCACGACATGTAACAAGGGAAA -3'
vYFP_URA_L	5'- GTGGTGGTTGGCAAATGACTAATTAAGTTAGTCAAGGCGC CATCCgagctcagtttatcattatcaa -3'
vYFP_URA_R	5'- CATTTC AATAAAAGTTCGTTTTATAAAAGTATAGTACTTGTGA GAGggtaccggccgcaaatta -3'

* In primers for gene deletion with *LEU2* and *URA3*, capital letters are ORF flanking sequences and lowercase letters are used for amplifying *LEU2* or *URA3* from plasmids.

Table S13. Fitness of single-deletion yeast strains relative to BY4743 in the first set of yeast experiment.

ORF name	Gene name	Replacement by <i>LEU2</i>	Replacement by <i>URA3</i>
YAR002W	<i>NUP60</i>	0.952	0.991
YDL193W	<i>NUS1</i>	0.946	0.968
YFL039C	<i>ACT1</i>	0.906	0.891
YHR143W-A	<i>RPC10</i>	0.953	0.929
YIL142W	<i>CCT2</i>	0.938	0.918
YJR123W	<i>RPS5</i>	0.888	0.859
YKL006W	<i>RPL14A</i>	0.898	0.898
YPR181C	<i>SEC23</i>	0.908	0.921

Table S14. Standard deviation of flow cytometry cell counting repeats of the same competition samples between BY4742 and BY4742-vYFP.

Sample #	P_0	Technical repeat #	P_1	BY4742 fitness relative to BY4742-vYFP	Standard deviation between technical repeats
1	69.644	1	68.198	1.0085	0.0004
		2	68.293	1.0079	
2	58.855	1	56.988	1.0096	0.0012
		2	57.314	1.0079	
3	48.813	1	46.971	1.0093	0.0010
		2	46.681	1.0107	
4	39.264	1	37.480	1.0095	0.0006
		2	37.643	1.0086	
5	30.473	1	28.743	1.0104	0.0007
		2	28.572	1.0115	
Mean					0.00078

Table S15. Biological repeats of competitions between BY4743 and BY4742-vYFP

Repeat #	P_0	P_1	BY4343 fitness relative to BY4742-vYFP
1	49.088	25.246	1.039
2	46.816	24.705	1.039
3	47.689	25.671	1.037
4	47.987	24.318	1.042
5	49.037	25.692	1.037
6	49.590	26.463	1.035
7	47.997	25.227	1.038
8	49.464	26.400	1.035
9	48.413	25.576	1.037
Average			1.038
Standard deviation			0.0021

Table S16. Fitness values of different colonies from the same gene replacement experiment.

Marker gene	Deleted gene	Repeats #	Fitness relative to BY4742- vYFP	Standard deviation
<i>URA3</i>	<i>HO</i>	1	1.011	0.0020
		2	1.016	
		3	1.015	
		4	1.014	
		5	1.016	
<i>LEU2</i>	<i>HO</i>	1	1.003	0.0029
		2	1.006	
		3	1.001	
		4	0.999	
		5	1.005	
<i>URA3</i>	<i>ACT1</i>	1	0.889	0.0038
		2	0.888	
		3	0.895	
Weighted average standard deviation				0.0029

Table S17. Fitness values of different colonies from double-deletion experiments in which the two *HO* alleles of BY4743 are replaced by *URA3* and *LEU2*, respectively.

Colony #	Fitness relative to BY4742-vYFP
1	1.014
2	1.015
3	1.016
4	1.018
5	1.018
Standard deviation	0.0016

Table S18. Relative fitness of BY4743 to BY4742-vYFP is stable over time. The two experiments were six months apart and the fitness difference is not significant ($P > 0.5$, two tailed t test).

	Fitness of BY4743 relative to BY4742-vYFP	
	Repeat 1	Repeat 2
Experiment 1	1.0367	1.0367
Experiment 2	1.0391	1.0364

Table S19. Fitness of single-deletion yeast strains relative to BY4742 in the second set of yeast experiment.

ORF name	Gene name	Replacement/insertion by <i>LEU2</i>	Replacement/insertion by <i>URA3</i>
YBR243C*	<i>ALG7</i>	0.898	0.932
YDR454C*	<i>GUK1</i>	0.903	0.993**
YLR088W*	<i>GAA1</i>	0.831	0.892
YOL078W*	<i>AVO1</i>	0.929	0.988**
YJL029C	<i>VPS53</i>	0.901	0.861
YMR307W	<i>GAS1</i>	0.688	0.726
YNL080C	<i>EOS1</i>	0.798	0.879
YOL064C	<i>MET22</i>	0.865	0.885
YGR157W	<i>CHO2</i>	0.920	0.967

* Essential genes for which DAMP strains were made.

** Fitness reduction is too small for measuring epistasis.