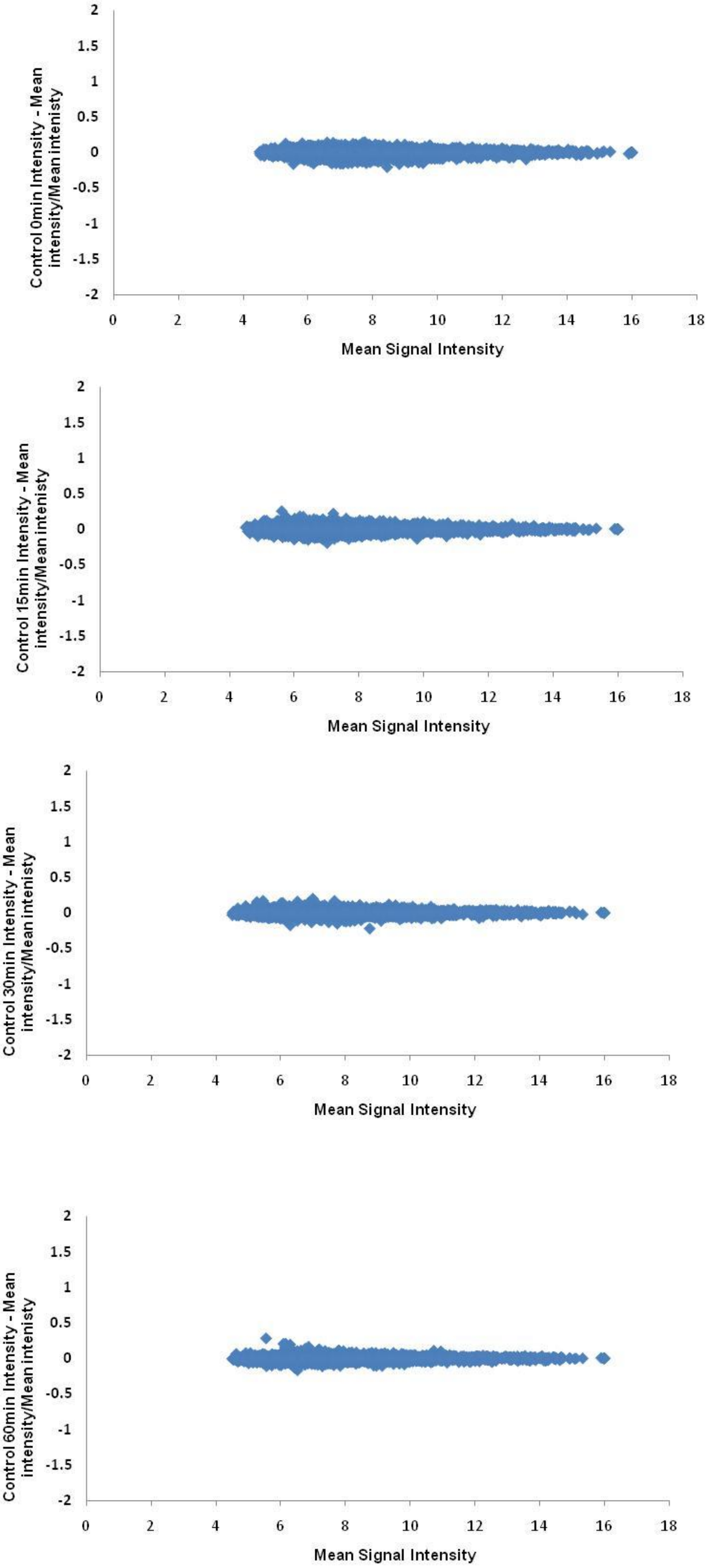
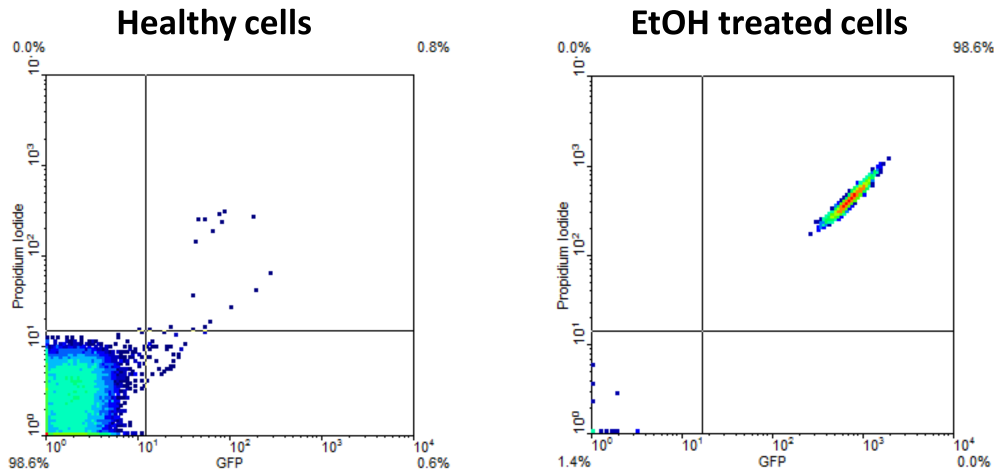


Supplementary figure 1

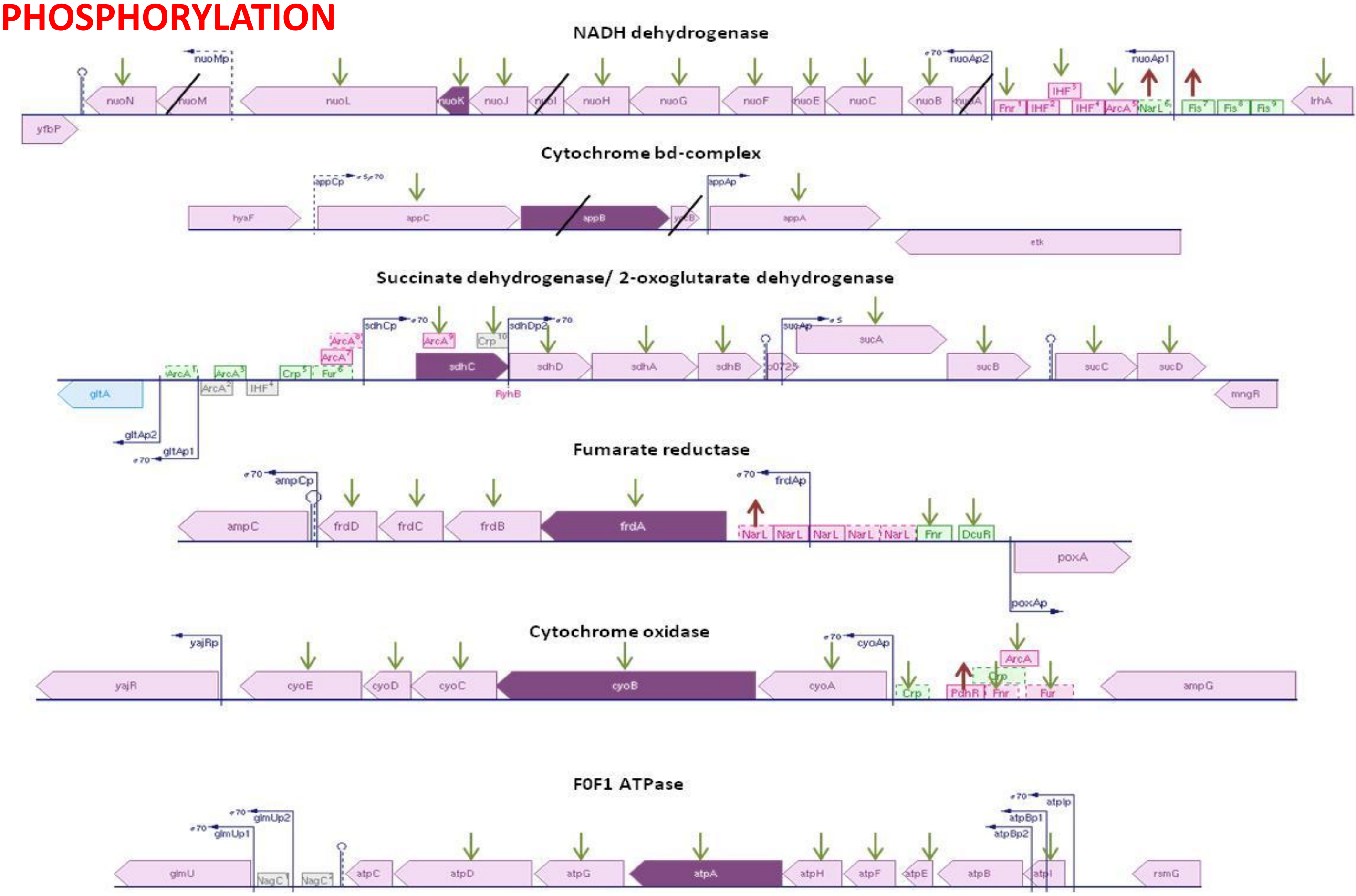


Healthy cells

EtOH treated cells

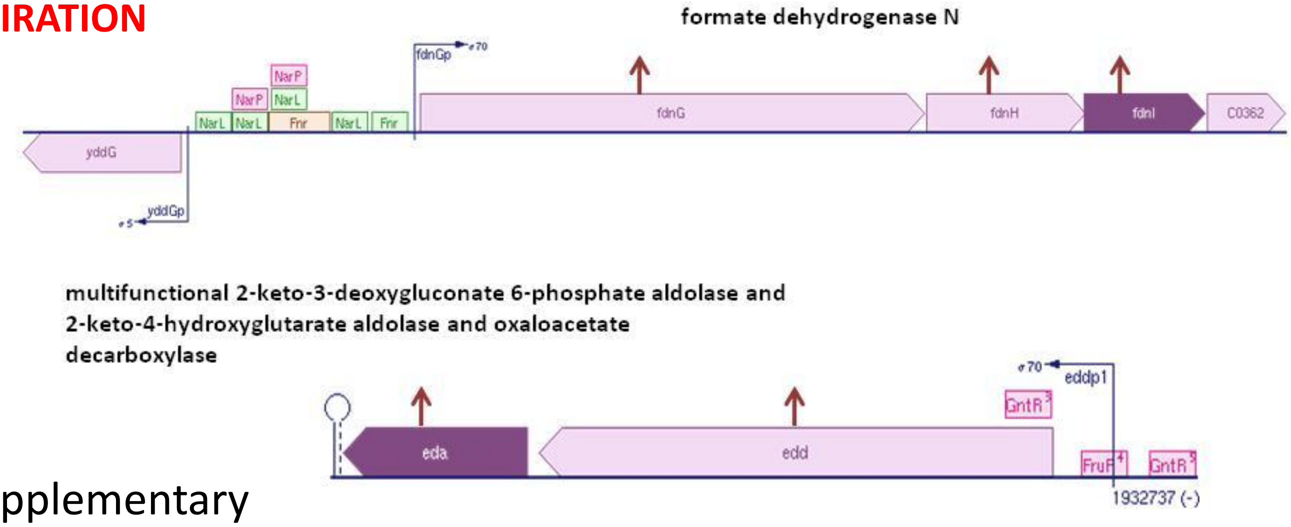
Supplementary figure 2

OXIDATIVE PHOSPHORYLATION



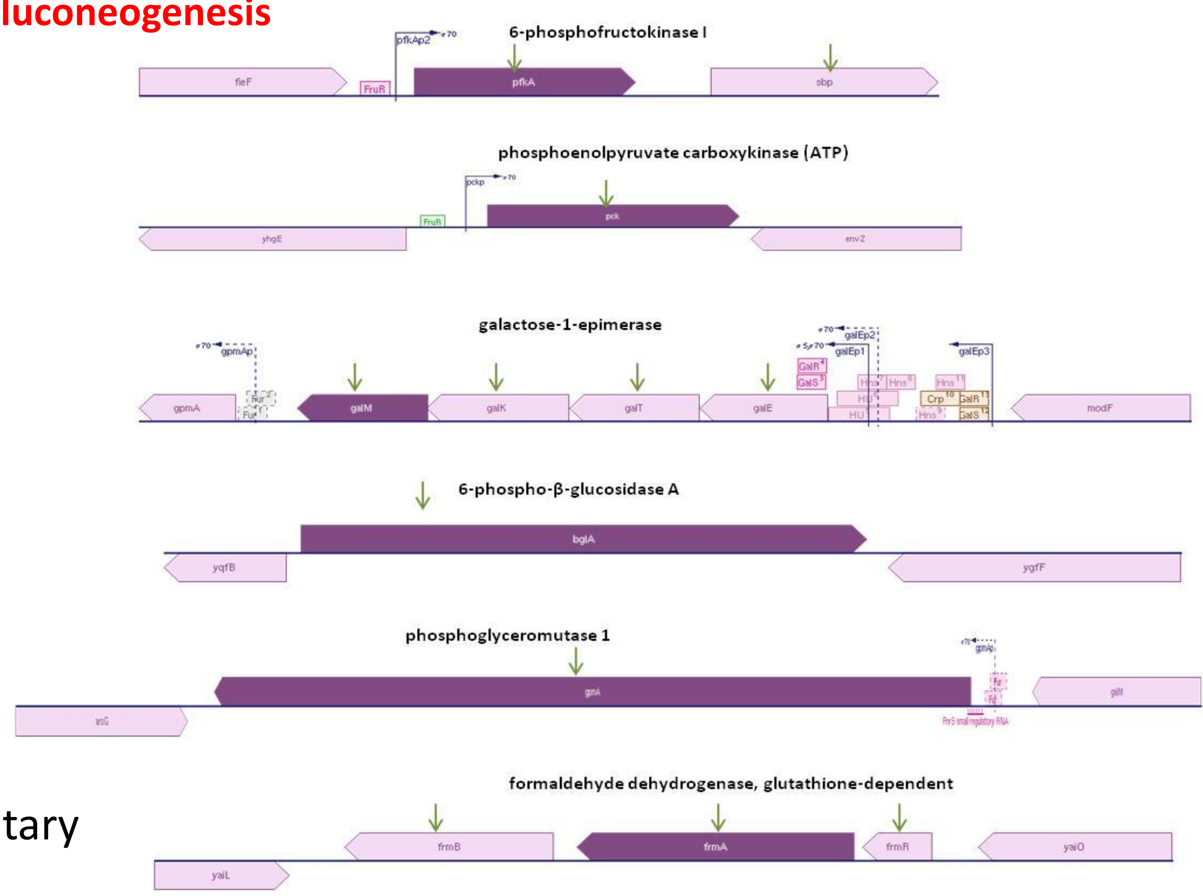
Supplementary figure 3 (A)

ANAEROBIC RESPIRATION



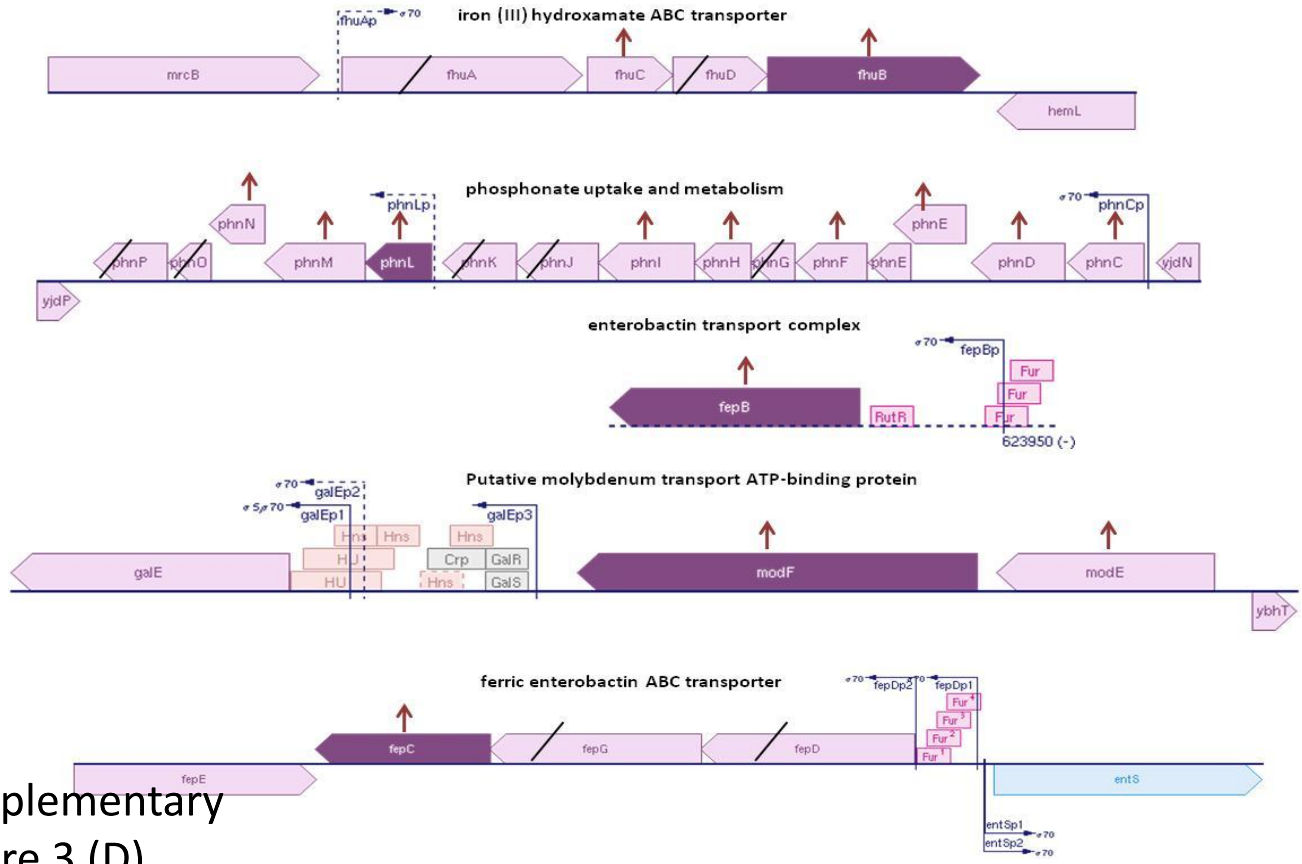
Supplementary figure 3 (B)

Glycolysis/Gluconeogenesis

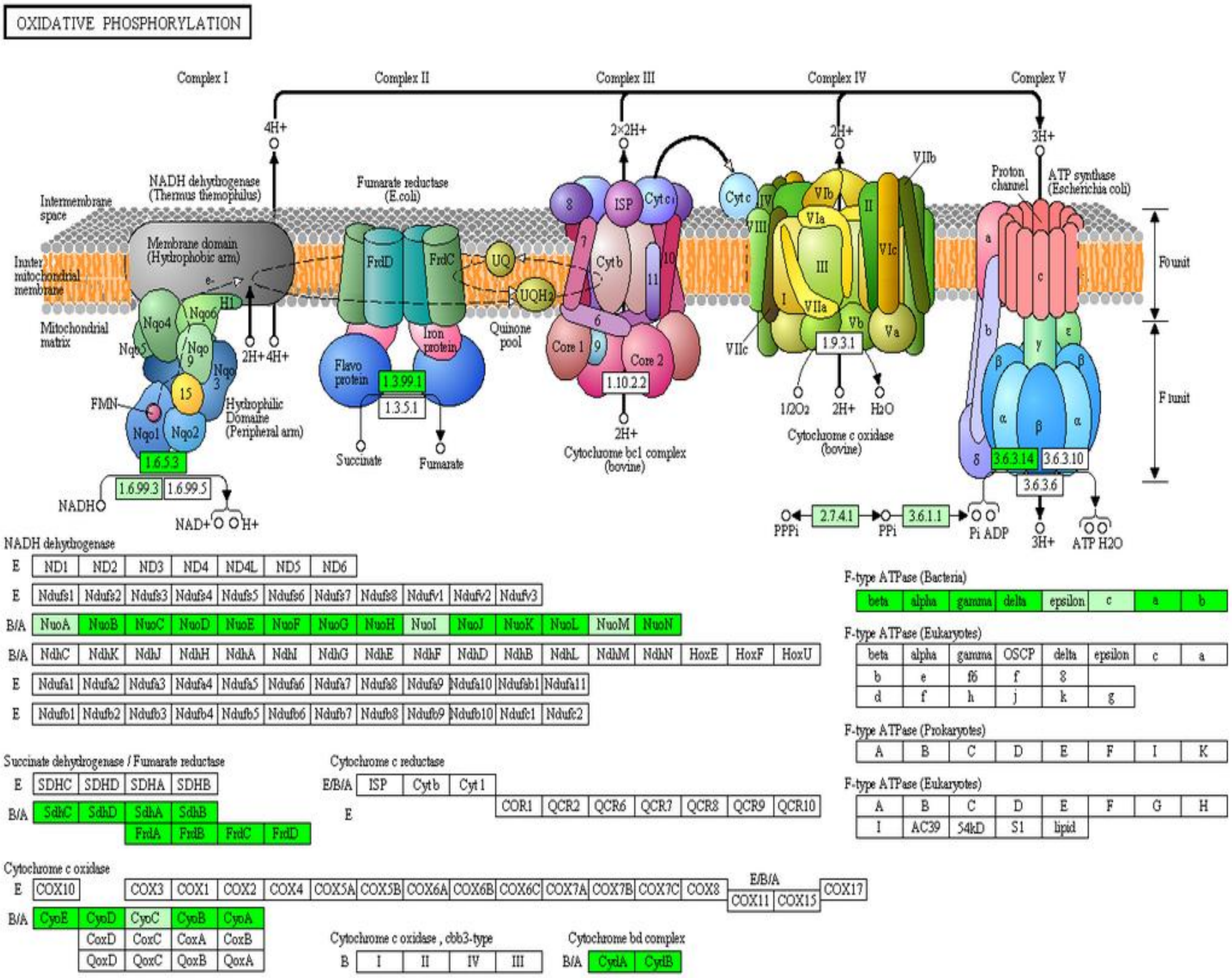


Supplementary figure 3 (C)

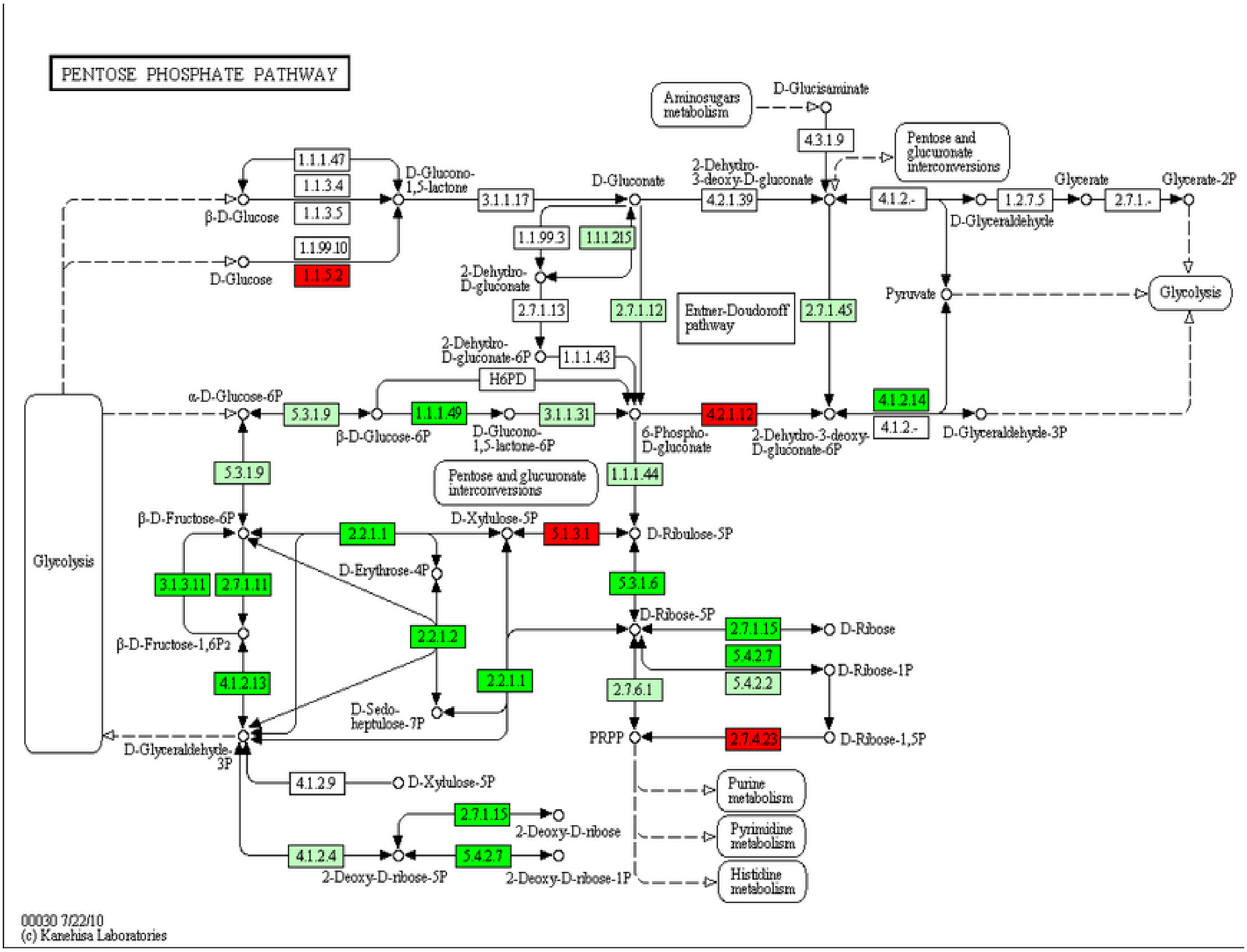
ABC Transporters



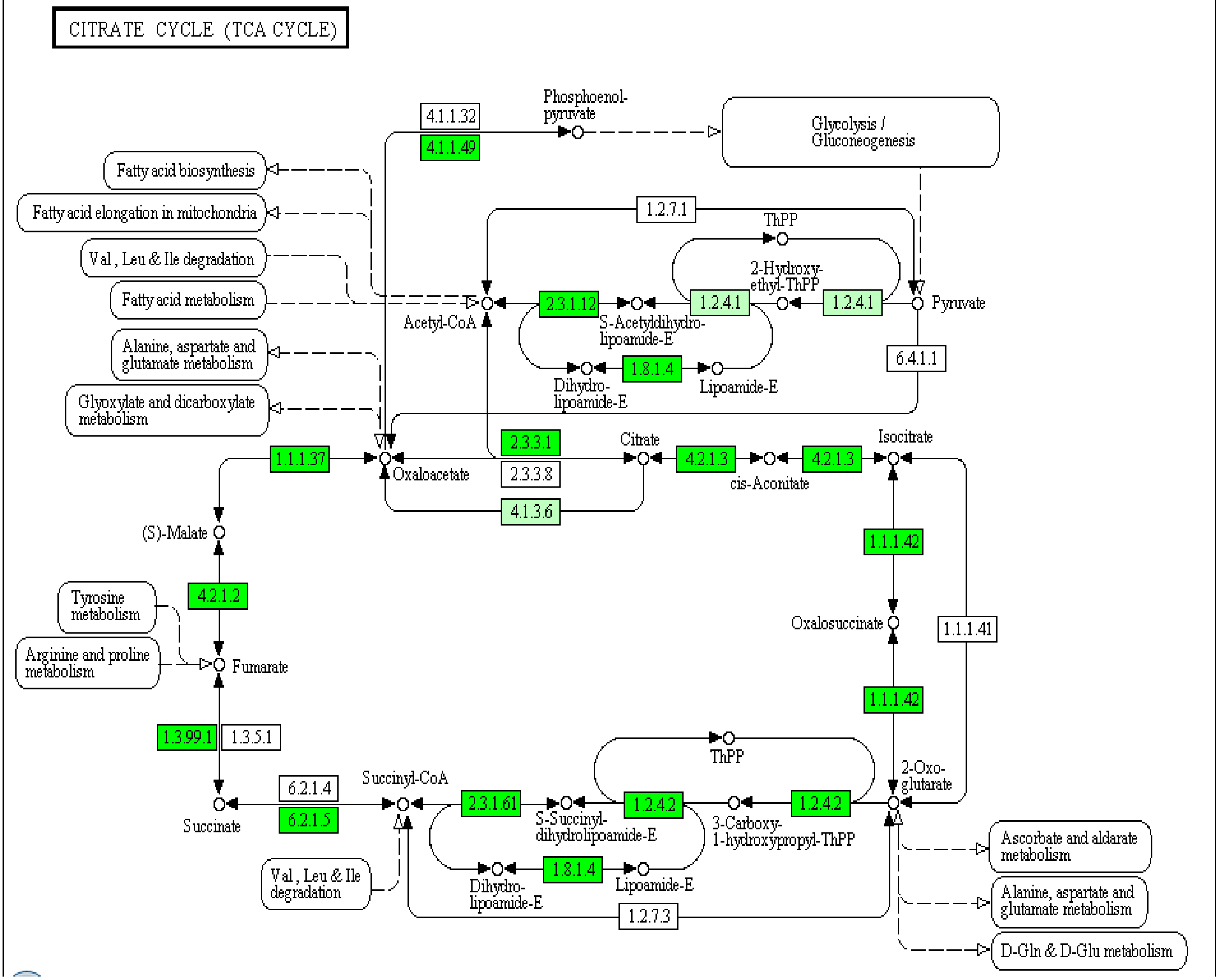
Supplementary figure 3 (D)



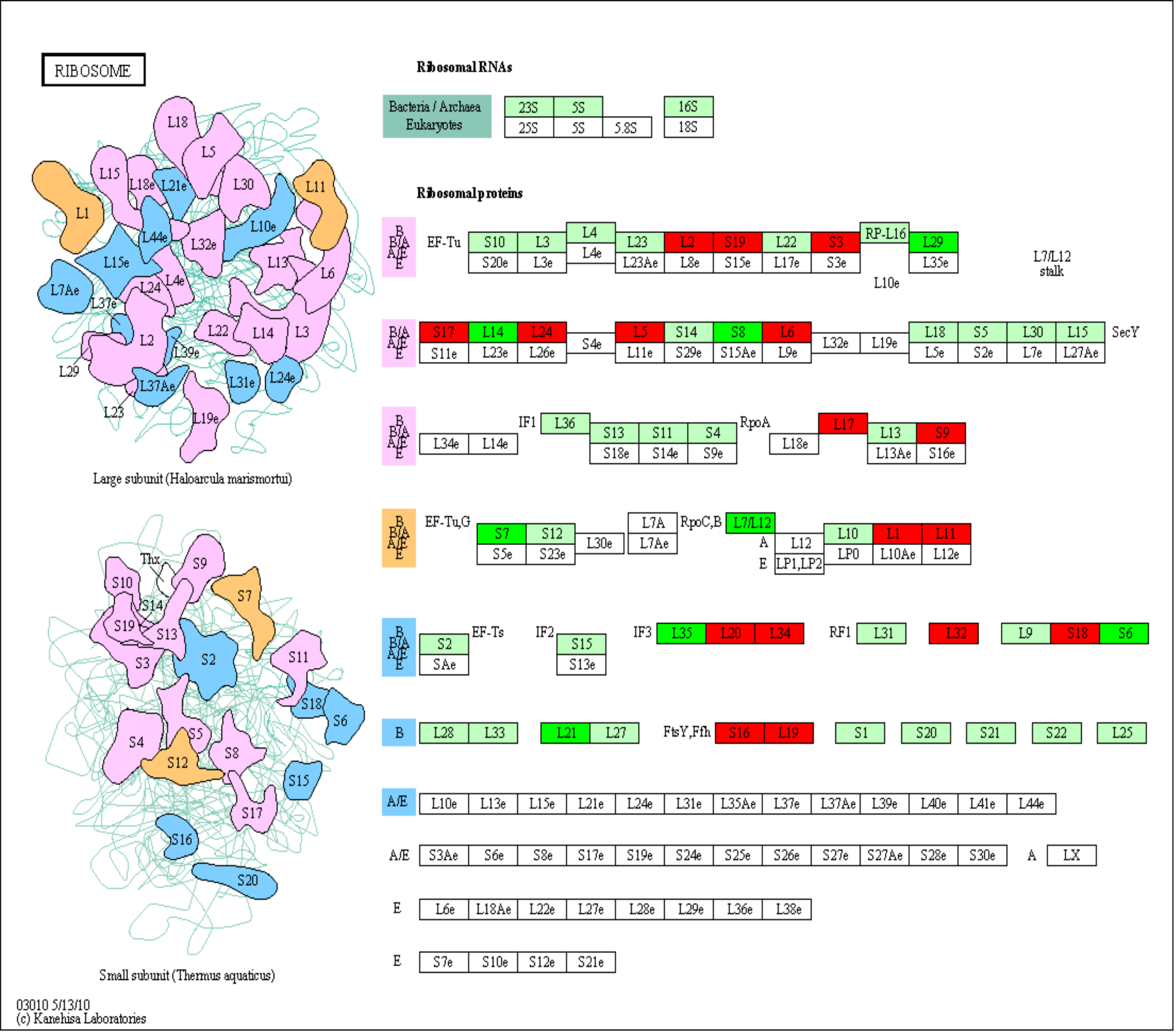
Supplementary figure 4 (1)



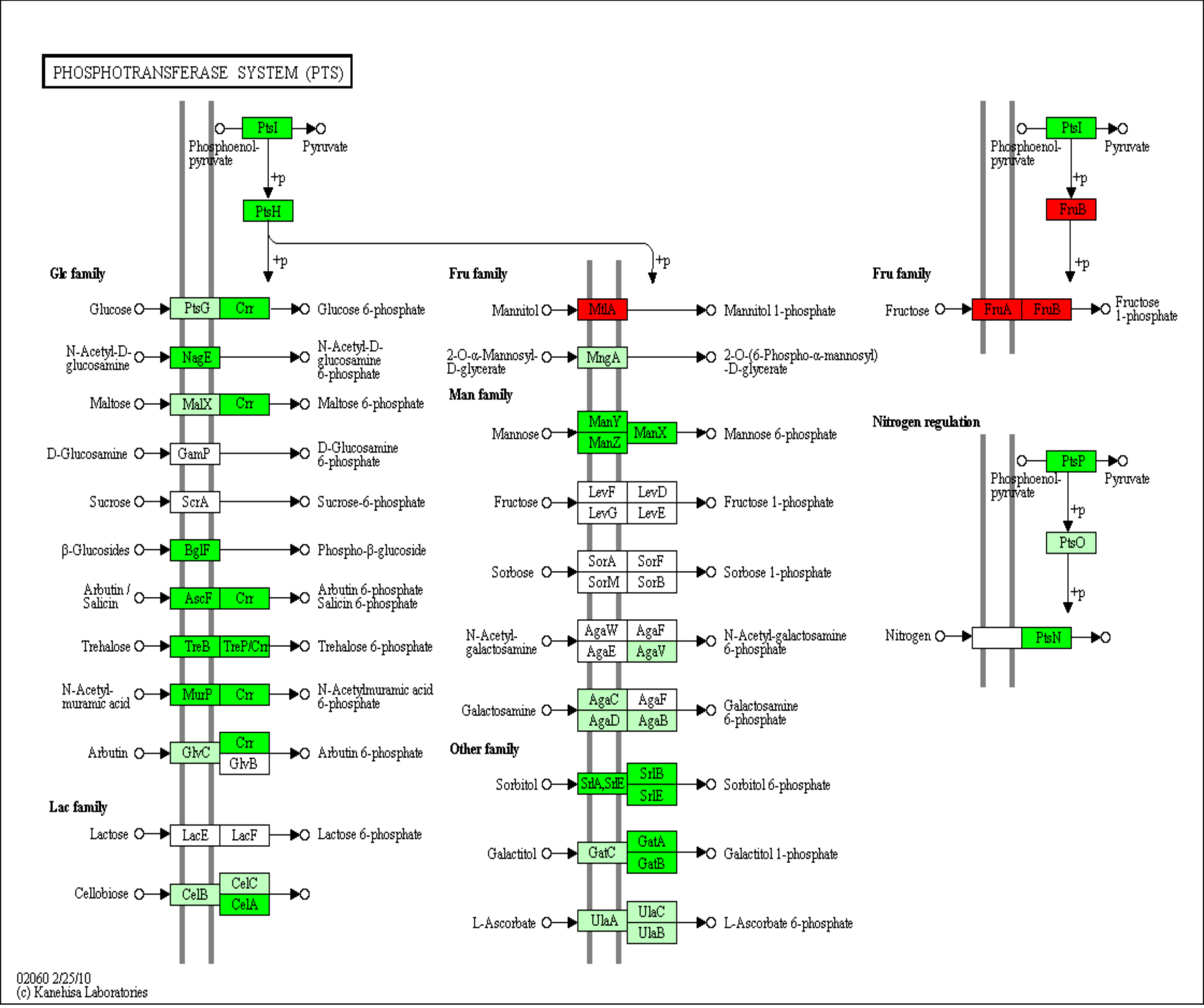
Supplementary figure 4 (2)



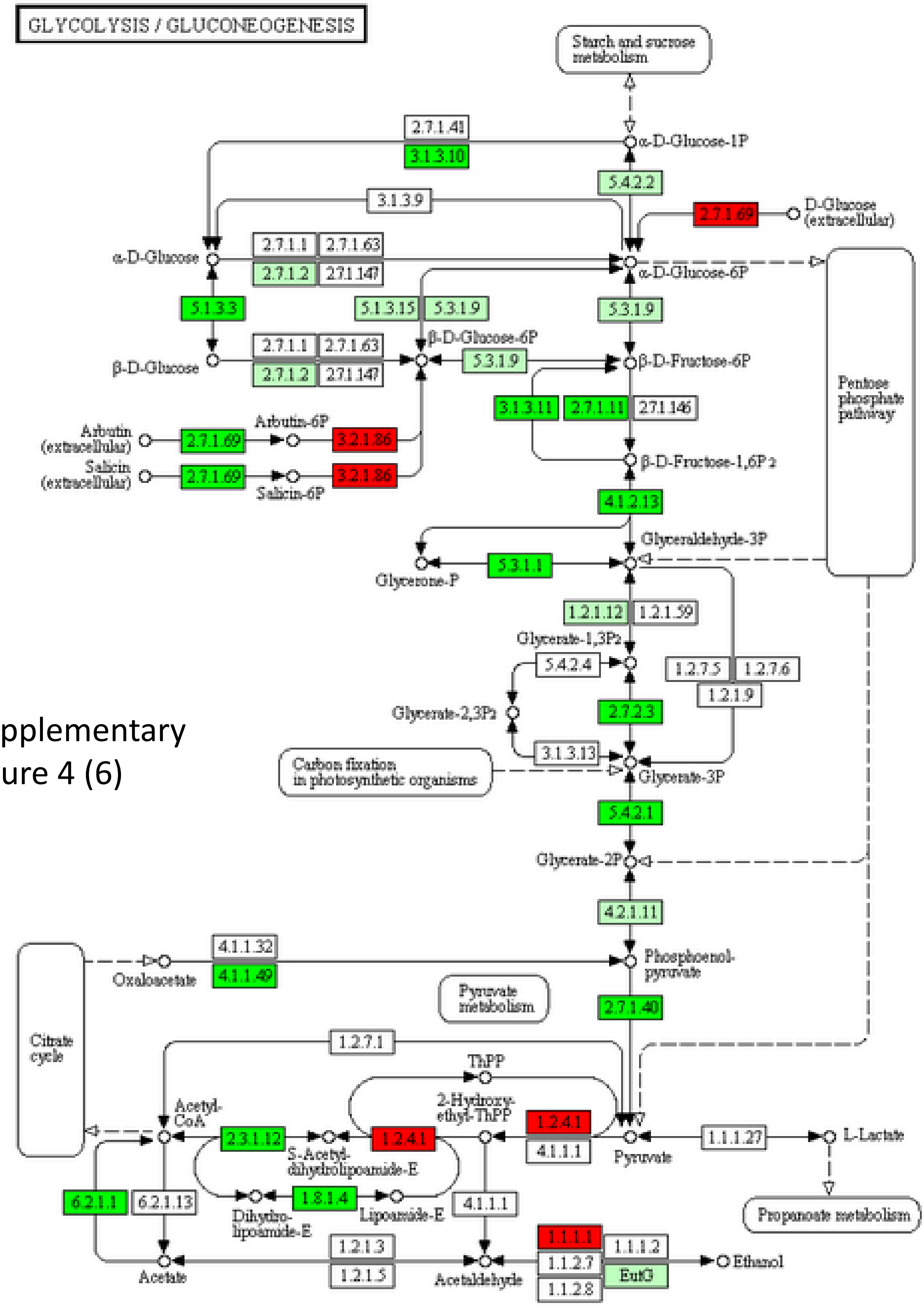
Supplementary figure 4 (3)



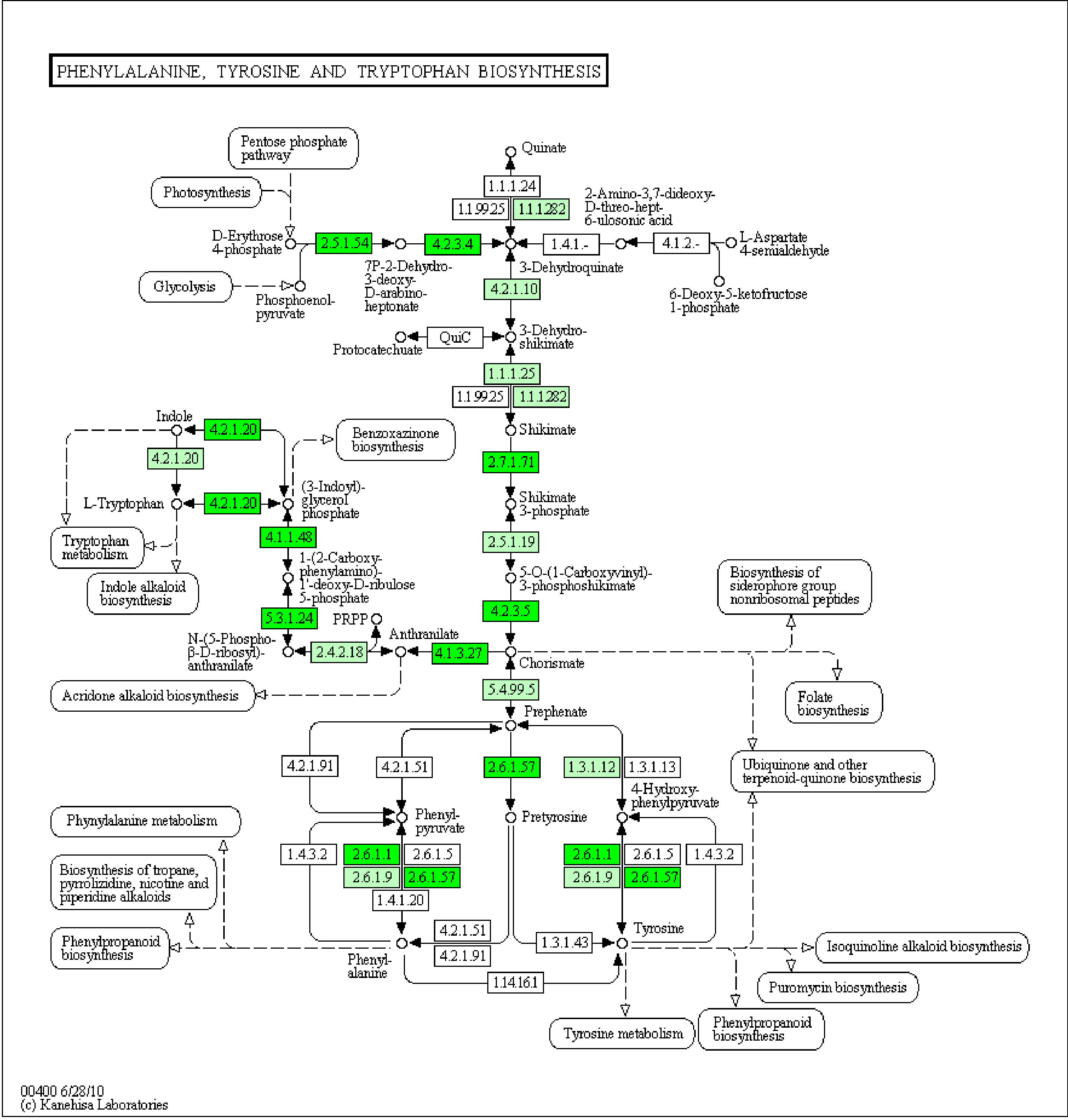
Supplementary figure 4 (4)



Supplementary figure 4 (5)

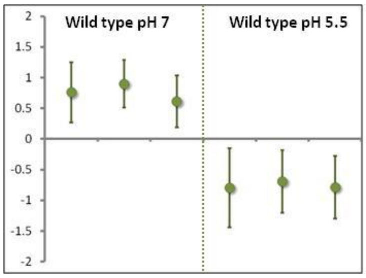


Supplementary figure 4 (6)



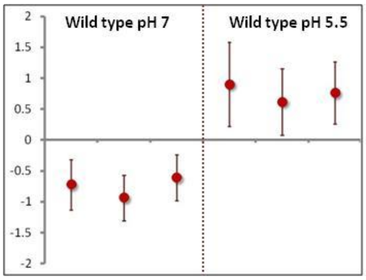
Supplementary figure 4 (7)

SAM 10% FDR - 973 negative genes



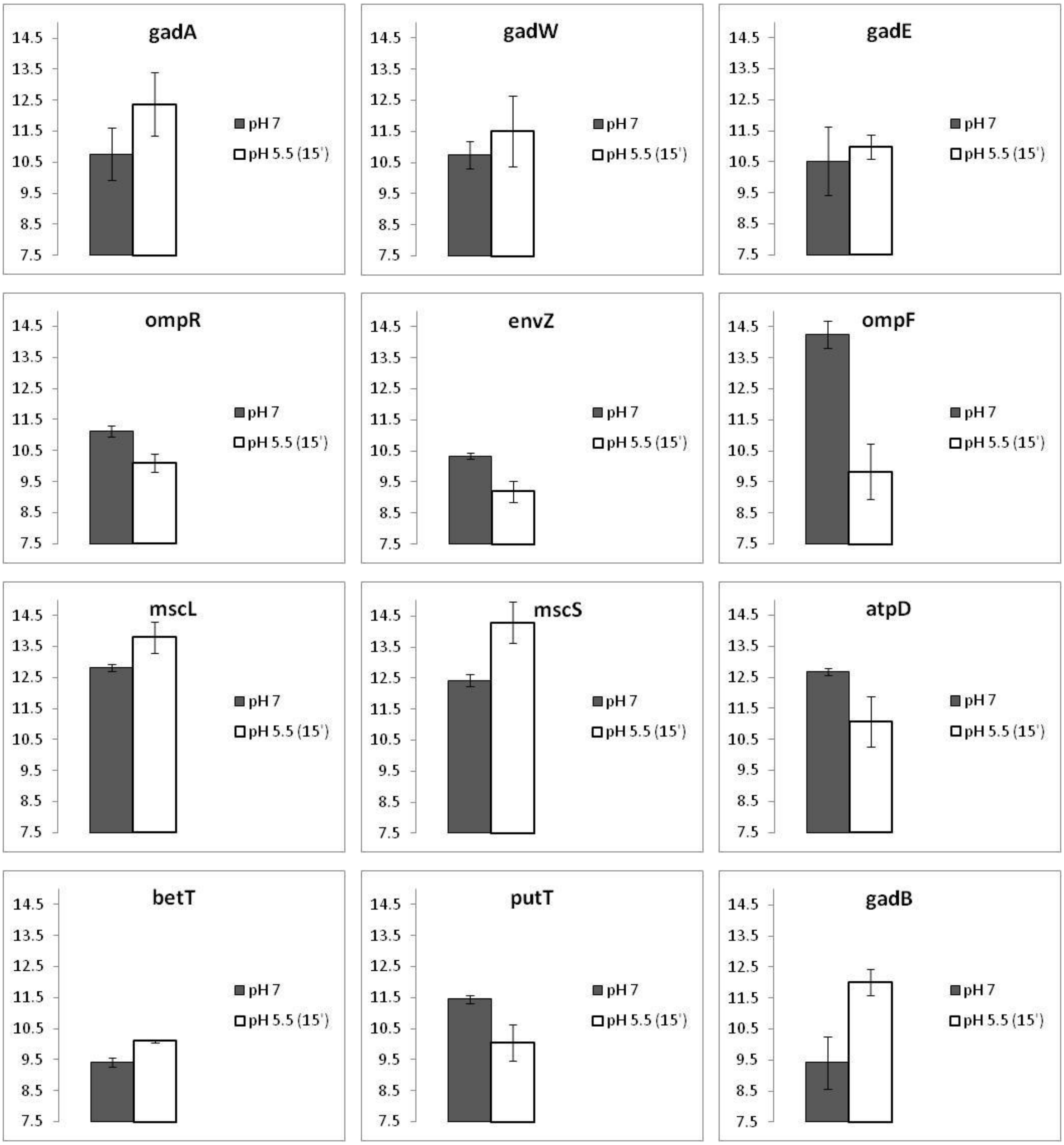
Category	Term	Count	PValue	Benjamini	ASRS	AFI
SP_PIR_KEYWORDS	transport	115	6.76E-20	9.16E-19	crp, atpD, phoQ, phoP, lrp, cysB, evgA, rpoS, atpC	
KEGG_PATHWAY	eum03010:Ribosome	25	1.79E-16	1.26E-13		
KEGG_PATHWAY	ecx00230:Purine metabolism	28	6.69E-15	8.07E-13		
KEGG_PATHWAY	ecc00240:Pyrimidine metabolism	24	9.95E-16	2.12E-13		
KEGG_PATHWAY	ecv00970:Aminoacyl-tRNA biosynthesis	16	3.15E-13	2.14E-11		
KEGG_PATHWAY	ecq00250:Alanine, aspartate and glutamate metabolism	15	3.00E-11	1.24E-09		
KEGG_PATHWAY	ecr00061:Fatty acid biosynthesis	10	1.07E-09	3.25E-08		
SP_PIR_KEYWORDS	cell cycle	20	6.08E-12	5.44E-11		
KEGG_PATHWAY	ecg00190:Oxidative phosphorylation	16	2.63E-09	7.43E-08		
KEGG_PATHWAY	eco00270:Cysteine and methionine metabolism	12	1.56E-07	2.82E-06		
KEGG_PATHWAY	ecr02010:ABC transporters	32	1.54E-08	3.74E-07		
KEGG_PATHWAY	ecg00010:Glycolysis / Gluconeogenesis	16	4.79E-10	1.56E-08		
KEGG_PATHWAY	ecd00620:Pyruvate metabolism	23	1.97E-17	3.35E-14		
KEGG_PATHWAY	eck00500:Starch and sucrose metabolism	11	6.57E-06	8.58E-05		
KEGG_PATHWAY	ecg02020:Two-component system	26	2.78E-08	6.05E-07		
KEGG_PATHWAY	eco00520:Amino sugar and nucleotide sugar metabolism	16	8.13E-09	2.09E-07		
KEGG_PATHWAY	ect00260:Glycine, serine and threonine metabolism	11	4.76E-06	6.35E-05		
KEGG_PATHWAY	ecj02060:Phosphotransferase system (PTS)	14	4.09E-07	6.67E-06		
KEGG_PATHWAY	ecr00770:Pantothenate and CoA biosynthesis	9	7.00E-06	9.07E-05		
KEGG_PATHWAY	ecx03440:Homologous recombination	10	1.06E-05	1.35E-04		

SAM 10% FDR - 898 positive genes



Category	Term	Count	PValue	Benjamini	ASRS	AFI
KEGG_PATHWAY	ecd02010:ABC transporters	36	4.19E-21	5.81E-18	evgS,	slp, gadA, yhiD, hdeA, hdeD
KEGG_PATHWAY	ecj02020:Two-component system	23	7.51E-12	6.51E-10	hdeA,	
KEGG_PATHWAY	ecd00330:Arginine and proline metabolism	12	6.43E-09	2.88E-07	gadB,	
GOTERM_BP_FAT	GO:0009061~anaerobic respiration	22	8.64E-09	2.66E-06	hdeD,	
KEGG_PATHWAY	eco00540:Lipopolysaccharide biosynthesis	9	4.27E-07	1.44E-05	adiC,	
SP_PIR_KEYWORDS	ion transport	22	8.98E-08	1.10E-06	cadC,	
SP_PIR_KEYWORDS	amino-acid transport	18	2.30E-12	5.63E-11	cadA,	
KEGG_PATHWAY	ecd00052:Galactose metabolism	8	1.69E-05	5.09E-04	gadA,	
KEGG_PATHWAY	ecg00053:Ascorbate and aldarate metabolism	6	2.54E-05	7.04E-04	gadC,	
KEGG_PATHWAY	ecg00630:Glyoxylate and dicarboxylate metabolism	7	3.49E-04	0.0073022	ydeO	

Supplementary
figure 5



Supplementary
figure 6

C-BOX

cyoA: cytochrome bo terminal oxidase subunit II

ACAGCTTCTTAAAATCAACCTGATATGTTTTGCAACATATGTGACCTGGCAGCCAAATCCAAGTAACAGG
TGAA-CAT-T

Start	End	Strand	PWM Score(s)	SEP Score	ATG-Distance	Location
450955	450964	+	12.18	-6.68	121	intergenic

argQ: tRNAargQ

AGTAATCCTCCCGGATGCACCATCTCTTACTTGATACGGCTTTAGTAGCG ... **(130)...**
TCGAATCCTCCCGGATGCACCATCTCTTACTTGATATGGCTTTAGTAGC
TGAA-CAT-T

Start	End	Strand	PWM Score(s)	SEP Score	ATG-Distance	Location
2815943	2815952	+	12.04	-7.01	61	intergenic
2816217	2816226	+	12.04	-6.84	335	coding region

argZ: tRNAargZ

TTCGAATCCTCCCGGATGCACCATATTCTACGTACTTTCAGCGATGAAGG ... **(124)...**
CCCGGATGCACCATCTCTTACTTGATACGGCTTTAGTAGCGGTATC
TGAA-CAT-T

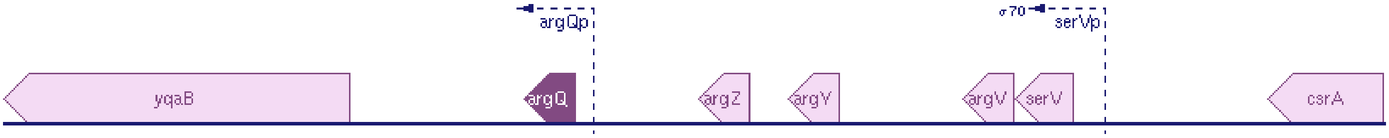
Start	End	Strand	PWM Score(s)	SEP Score	ATG-Distance	Location
2816217	2816226	+	12.04	-6.84	60	coding region
2816358	2816367	+	12.04	-6.96	201	coding region

Supplementary figure 7 (1)

argY: tRNAargY

CCTCCCGGATGCACCATCTCTTACTTGATACGGCTTTAGTAGCGGTATCA
TGAA-CAT-T

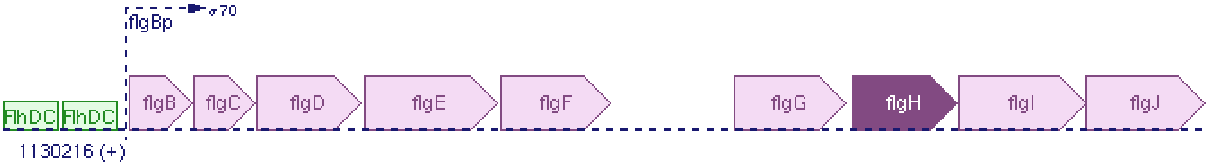
Start	End	Strand	PWM Score(s)	SEP Score	ATG-Distance	Location
2816358	2816367	+	12.04	-6.96	62	intergenic



flgH: flagellar L-ring protein

AACTGGTCAATATGATTCAGGTGCAACGCGCTTACGAAATCAACAGTAAA ... (50)...
CCACCGATCAGATGCTGCAAAAACTGACGCAACTCTAAGGCTTAACCGGT
TGAA-CAT-T

Start	End	Strand	PWM Score(s)	SEP Score	ATG-Distance	Location
1134706	1134715	+	12.16	-7.2	72	coding region
1134646	1134655	+	12.55	-7.55	132	coding region



Supplementary
figure 7 (2)

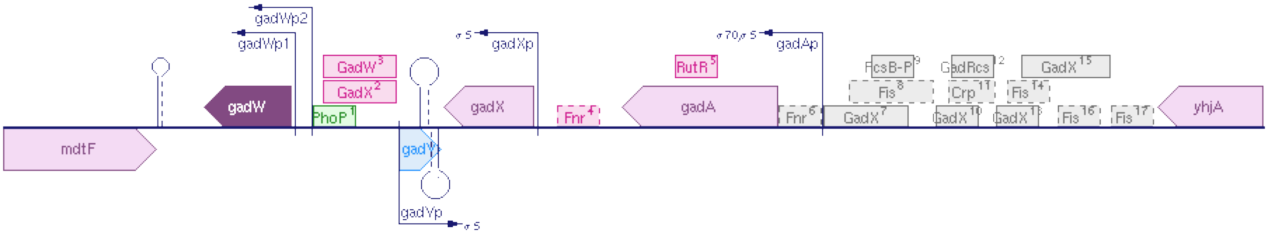
F-BOX

gadW: DNA-binding transcriptional dual regulator

ATATTCGTGCTGCATTTACTTATTATCAATTAACTGTTATGCAAAACTAC

TTTACA TTTT

Start	End	Strand	PWM Score(s)	SEP Score	ATG-Distance	Location
3662395	3662404	+	13.5	-6.45	147	intergenic



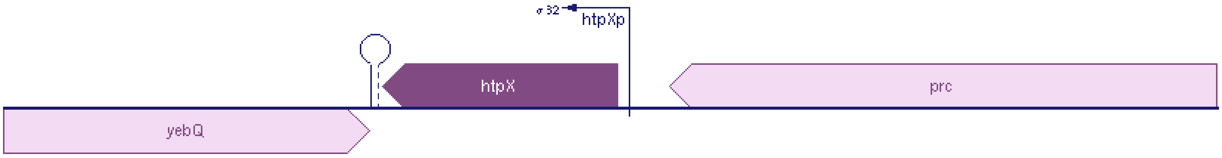
htpX: heat shock protein, integral membrane protein

CCGTAAATCAGATGCTACAAAATGTAAAGTTGTGTCTTTCTGGTGACTTA ... (100)...

TTTTGTTAAACTGAGGTAAAAATGAAAATTATG

T TTACATTTT

Start	End	Strand	PWM Score(s)	SEP Score	ATG-Distance	Location
1910603	1910612	-	13.5	-6.42	3	intergenic
1910713	1910722	-	14.09	-7	113	intergenic



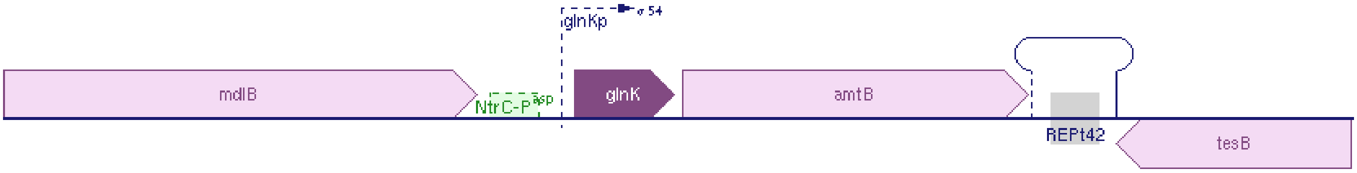
Supplementary figure 7 (3)

glnK: nitrogen regulatory protein GlnK

ATTTCATCGTTGGTGCAAAAATGTAACGCACTGTGCACTGTCATAGTGCGTTTTCATTTTCAAACTTCTT

TTTACATTTT **TTTACATTTT**

Start	End	Strand	PWM Score(s)	SEP Score	ATG-Distance	Location
471669	471678	-	13.48	-7.01	144	intergenic
471702	471711	+	13.5	-7.01	111	intergenic



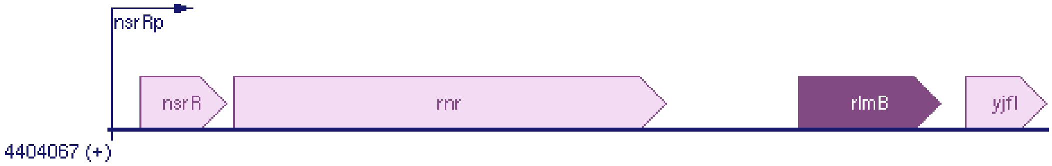
yjfH (rlmB): 23S rRNA 2'-O-ribose G2251 methyltransferase

TCGTCAGGTCGGTAAAAAGGTAAACTTTGAGCCAGACAGC GCCTTCCGCG ... **(310)**...

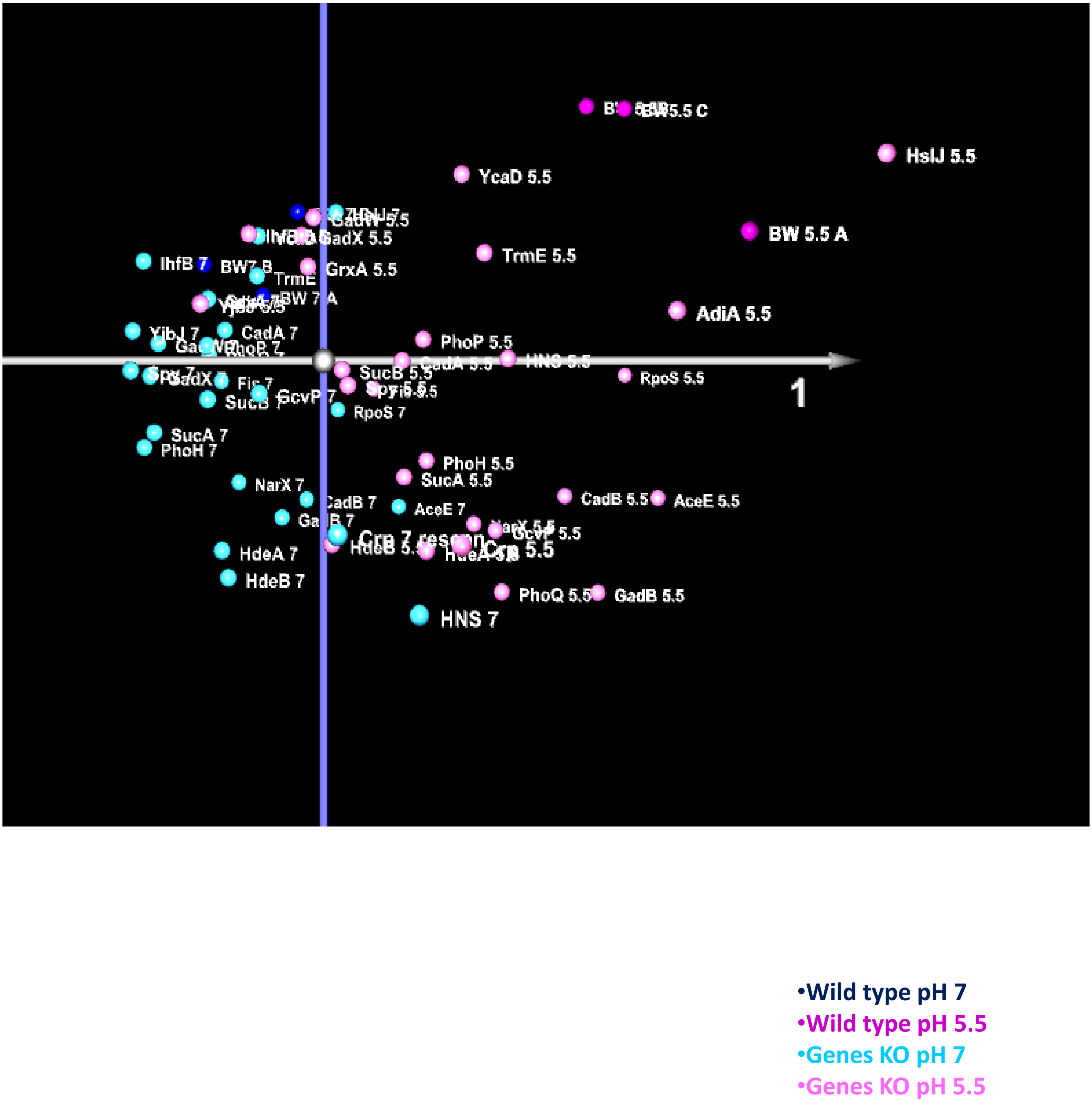
TAGTGCATCAGGCAAAACGTAAACAACGAGTACATTA ATG

TTTACATTTT

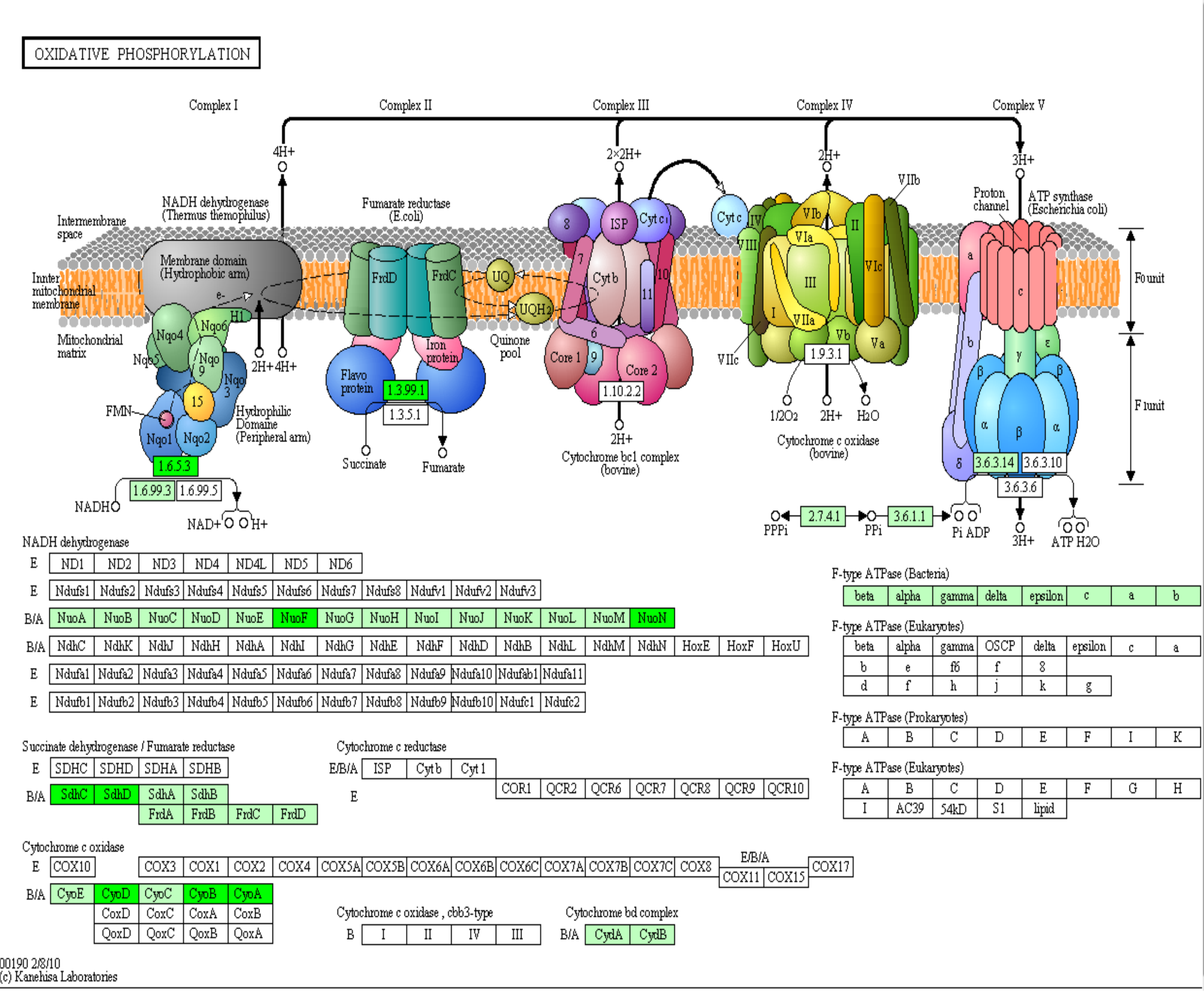
Start	End	Strand	PWM Score(s)	SEP Score	ATG-Distance	Location
4406507	4406516	-	13.59	-7.44	337	coding region
4406829	4406838	-	13.59	-7	15	intergenic



Supplementary figure 7 (4)



Supplementary figure 8



NADH dehydrogenase

E	ND1	ND2	ND3	ND4	ND4L	ND5	ND6										
E	Ndufs1	Ndufs2	Ndufs3	Ndufs4	Ndufs5	Ndufs6	Ndufs7	Ndufs8	Ndufv1	Ndufv2	Ndufv3						
B/A	NuoA	NuoB	NuoC	NuoD	NuoE	NuoF	NuoG	NuoH	NuoI	NuoJ	NuoK	NuoL	NuoM	NuoN			
B/A	NdhC	NdhK	NdhJ	NdhH	NdhA	NdhI	NdhG	NdhE	NdhF	NdhD	NdhB	NdhL	NdhM	NdhN	HoxE	HoxF	HoxU
E	Ndufa1	Ndufa2	Ndufa3	Ndufa4	Ndufa5	Ndufa6	Ndufa7	Ndufa8	Ndufa9	Ndufa10	Ndufab1	Ndufa11					
E	Ndufb1	Ndufb2	Ndufb3	Ndufb4	Ndufb5	Ndufb6	Ndufb7	Ndufb8	Ndufb9	Ndufb10	Ndufc1	Ndufc2					

Succinate dehydrogenase / Fumarate reductase

E	SDHC	SDHD	SDHA	SDHB		
B/A	SdhC	SdhD	SdhA	SdhB		
			FrdA	FrdB	FrdC	FrdD

Cytochrome c reductase

E/B/A	ISP	Cyt b	Cyt 1						
E				COR1	QCR2	QCR6	QCR7	QCR8	QCR9	QCR10

Cytochrome c oxidase

E	COX10	COX3	COX1	COX2	COX4	COX5A	COX5B	COX6A	COX6B	COX6C	COX7A	COX7B	COX7C	COX8	COX17
E/B/A														COX11 COX15	
B/A	CyoE	CyoD	CyoC	CyoB	CyoA										
		CoxD	CoxC	CoxA	CoxB										
		QoxD	QoxC	QoxB	QoxA										

Cytochrome c oxidase , cbb3-type

B	I	II	IV	III

Cytochrome bd complex

B/A	CydA	CydB

F-type ATPase (Bacteria)

beta	alpha	gamma	delta	epsilon	c	a	b

F-type ATPase (Eukaryotes)

beta	alpha	gamma	OSCP	delta	epsilon	c	a
b	e	f6	f	8			
d	f	h	j	k	g		

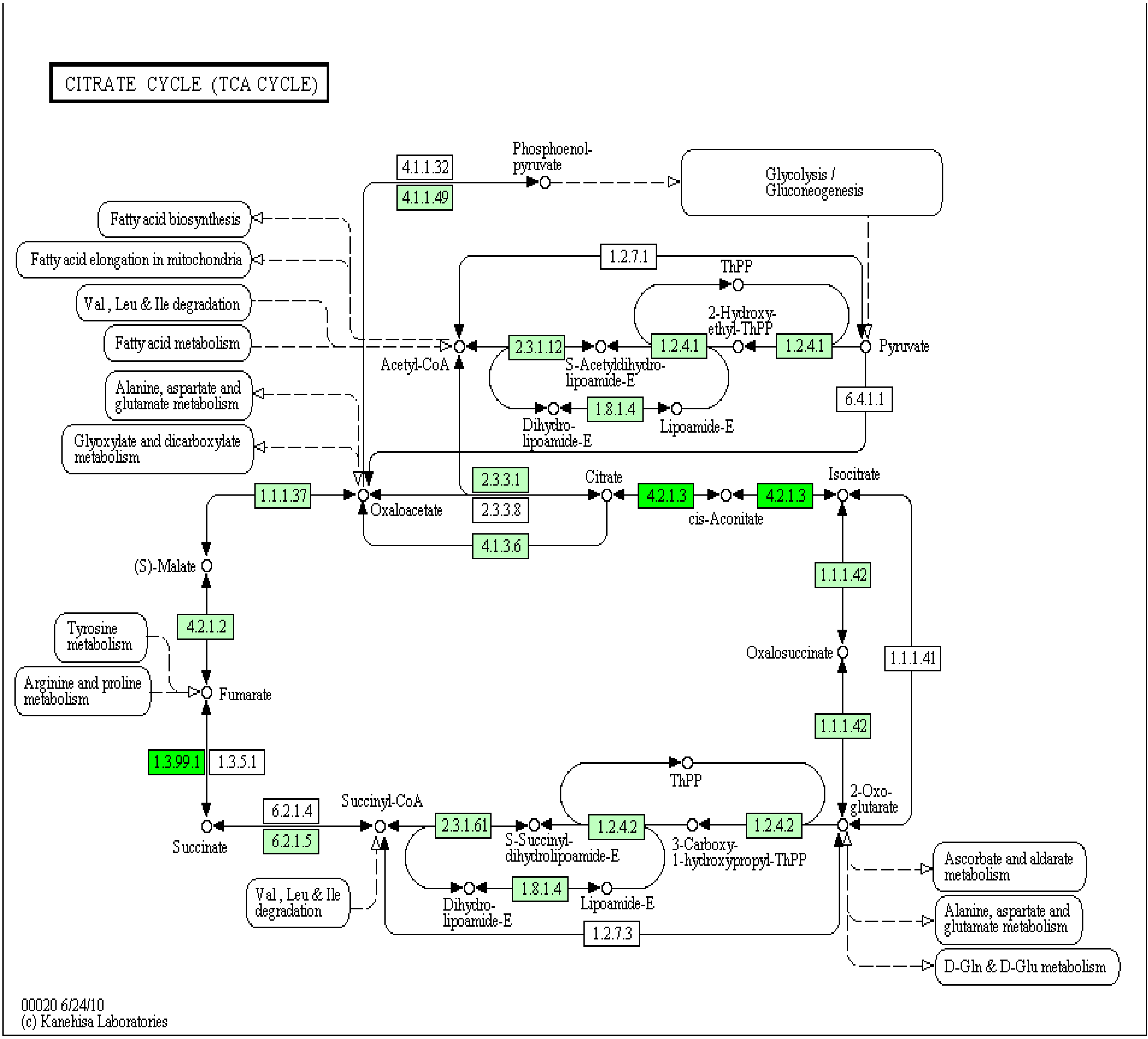
F-type ATPase (Prokaryotes)

A	B	C	D	E	F	I	K

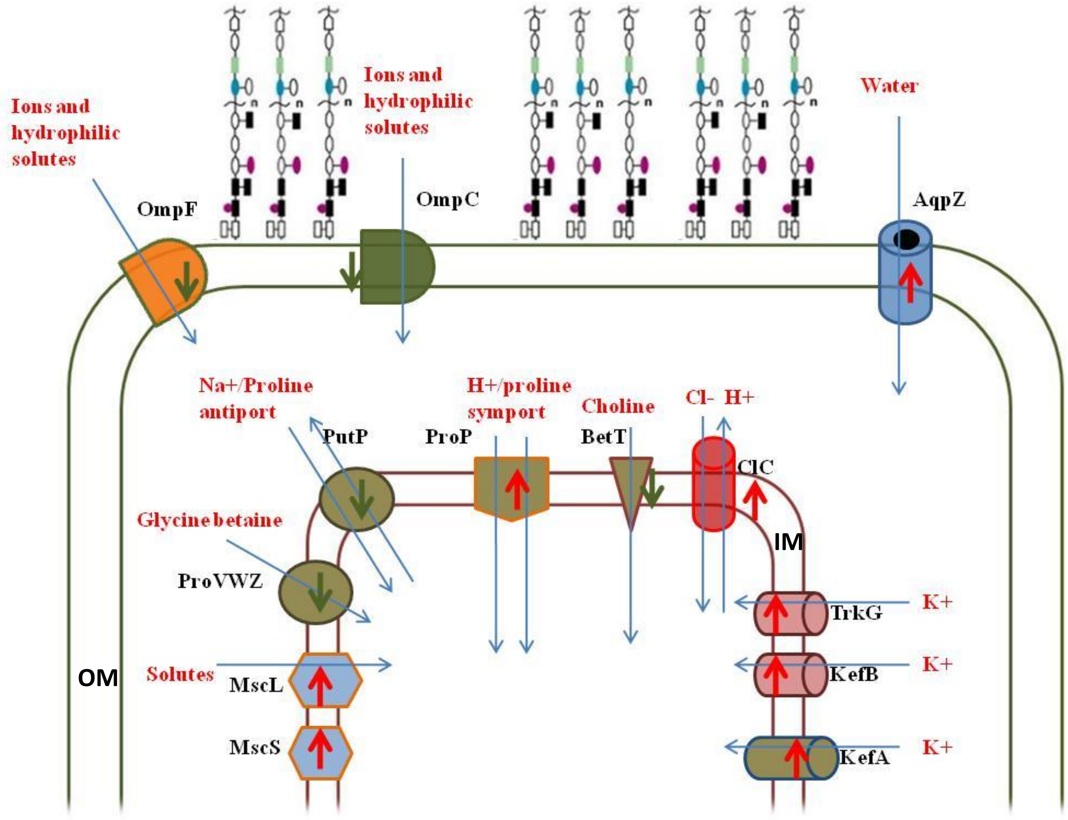
F-type ATPase (Eukaryotes)

A	B	C	D	E	F	G	H
I	AC39	54kD	S1	lipid			

Supplementary figure 9 (1)



Supplementary figure 9 (2)



Supplementary figure 10

Selected gene DOWN-regulated	**Function**
crp	cAMP receptor protein
fliA	RNA polymerase sigma factor for flagellar operon
fnr	Fumarate and nitrate reduction regulatory protein
gadE	Transcriptional regulator of genes involved in acid resistance
gcvP	Glycine dehydrogenase
hemE	Oxygen sensor protein
ihfA	Integration host factor subunit a
ihfB	Integration host factor subunit b
osmC	Osmotically-inducible protein C
phoH	Phosphate starvation-inducible protein
phoP	Member of the two-component regulatory system phoQ/phoP involved in adaptation to low Mg2+
phoQ	Member of the two-component regulatory system phoQ/phoP involved in adaptation to low Mg2+
pyrF	Orotidine 5'-phosphate decarboxylase
rpoS	RNA polymerase sigma factor for protection against external stresses
sucA	2-oxoglutarate dehydrogenase E1 component
sucB	Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex
Selected gene UP-regulated	**Function**
aceE	Pyruvate dehydrogenase E1 component
adiA	Biodegradative arginine decarboxylase
cadA	Lysine decarboxylase
cadB	Cadaverine/lysine antiporter
fis	Factor-for-inversion stimulation protein
gadB	Glutamate decarboxylase b
gadW	HTH-type transcriptional regulator of genes involved in acid resistance
gadX	HTH-type transcriptional regulator of genes involved in acid resistance
gcd	Quinoprotein glucose dehydrogenase
grxA	Glutaredoxin-1
hdeA	Chaperone-like acid stress protein
hdeB	Chaperone-like acid stress protein
hslJ	Heat shock protein
narJ	Nitrate reductase molybdenum cofactor assembly chaperone
narX	Nitrate/nitrite sensor protein
rpoD	The primary sigma factor of E. coli
spy	Spheroplast protein, could be invovled in zinc metabolism
trmE	tRNA modification GTPase
ycaD	Uncharacterized MFS-type transporter
ydeO	HTH-type transcriptional regulator of genes involved in acid resistance
yjbJ	Predicted stress response protein

Supplementary table 1

NOT ADAPTED	5' Acid	30' Acid	1 hour Acid	1 hr 30' Acid	2 hours Acid	2 hrs 30' Acid	3 hours Acid
ΔaceE	0.01462 5.4E-09	2.2E-10	8.69E-25 7.48E-25	1.25E-07	5E-21 2E-16	2E-18	8.4E-21 6.6E-12
ΔadiA	0.06828 0.06226	0.00876	0.003157 2.93E-10	0.087984	0.0094 2E-16	0.0018	0.37071 6.6E-12
ΔcadA	0.25351	6.2E-05 1.4E-23	2.8E-12 4.83E-27	0.000382	5E-09 6E-08	1E-07	0.00065 0.01769
ΔcadB	0.5429 2E-09	2.2E-13 0.00045	2.54E-16 0.001868	0.002945 0.166394	 9E-26	4E-29 9E-29	7.8E-15 1E-14
Δcrp	 1.4E-07	0.35534 1.4E-09	0.564475 4.86E-13	0.084494 7.68E-09	0.1488 0.0125	0.4457 0.0589	0.00061
Δfis	0.65632 1.8E-21	0.00231 0.01376	5.87E-08 1.89E-26	0.00017 2.53E-31	3E-06 3E-09	1E-07 1E-08	2.4E-06
ΔfliA	0.55652 0.25034		4.72E-05 0.000286	0.015941	0.0001 2E-29	 2E-32	0.00065 6.3E-20
Δfnr	0.0003 0.08236	3.8E-12	2.57E-14 0.0016	6.21E-13	1E-08 0.0003	1E-07	1.8E-07 0.02016
ΔgadB	0.31098 0.00032	0.35472	0.145061 1.85E-17	0.878042	0.6477 0.0045	0.0004	3.2E-08 0.56451
ΔgadE	0.32649	0.4173	0.891394	0.494995	0.7008 3E-17	0.7152	0.00051 8.6E-20
ΔgadW	0.01411	0.00876	0.001978	0.15801	0.0092	0.0067	0.36766
ΔgadX	0.06828	0.00359	0.000515	0.084125	0.007	0.0014	0.24428
Δgcd	0.09307	4.2E-06	5.6E-09	3.26E-07	3E-06	0.0004	0.00451
ΔgcvP	0.00701 0.7608	9.2E-11 2E-32	4.69E-18 1.94E-31	1.77E-09 5E-34	4E-17	1E-24	6.8E-18
ΔgrxA	0.04931 1	2.1E-07	1.03E-12 9.35E-20	8.31E-09	1E-12 5E-33	8E-07	0.03779
ΔhdeA	0.15328 2.2E-08	6.8E-07 6.7E-06	3.51E-11 1.85E-06	1.12E-11 9.77E-05	1E-19 2E-28	7E-21	4.6E-16 2.1E-19
ΔhdeB	0.04416 2E-09	2.5E-05 2.5E-08	9.72E-14 5.71E-11	3.91E-10 2.69E-07	2E-11 3E-05	4E-15 0.0014	5.1E-08
ΔhemE	0.56312	1	0.499132	0.169731	0.1157 1E-07	0.0006 1E-16	0.2909
Δhns	0.09307 1.1E-29	0.00046 2.2E-06	1.76E-07 2.96E-10	5.81E-07 5.62E-08	4E-10	1E-10	
ΔhslJ	1.1E-09 2.5E-05	2.4E-14	4.48E-18 2.11E-30	1.28E-14	2E-23 3E-07	2E-24 2E-08	4E-13
ΔihfA	0.67395		0.001048	0.000927	1E-05 1E-32		0.02347 7E-26
ΔihfB	0.01567	2.5E-29	1.27E-42	5.02E-32	1E-47	7E-33	
ΔnarJ	0.20821 0.12418		3.02E-08 1.89E-07	2.55E-07	1E-12		0.0001
ΔnarX	0.00016 0.25351	5E-08	6.6E-14 8.44E-11	4.37E-12	1E-14 2E-12	9E-15	1.2E-06 3.5E-08
ΔosmC	0.35778	0.00253	0.000461	0.000889	0.001 3E-21	8E-10	0.00615 3.3E-11
ΔphoB	0.28497	0.00033	9.38E-06	0.004672	0.0016	0.0011	0.77001
ΔphoH	0.00328 0.00027	6E-07 3.2E-05	2.58E-12 2.53E-05	2.72E-10 0.014569	8E-13	8E-14	5.4E-08
ΔphoP	0.0034 6.5E-13	4.2E-12 5.8E-18	1.02E-28 1.65E-22	3.16E-24 2.59E-17	2E-29 0.0019	5E-28 2E-30	1.6E-19
ΔphoQ	0.03514 0.00019	1E-10 1.9E-20	2.27E-17 3.35E-22	3.86E-07 1.21E-14	3E-28 6E-26	3E-32	8.7E-21
ΔrpoD	0.04796 0.26228	0.0024	4.76E-06 6.2E-08	2.85E-05	1E-06 2E-26	0.0002	0.35803
ΔrpoS	0.00062 8.1E-05	2.4E-16	9.71E-40 1.98E-33	2.32E-34	9E-37 0.0004	2E-38	2.8E-25 0.28719
Δspy	5.1E-08 0.00021	5.3E-14	2.17E-18 2.62E-18	7.25E-26	1E-26 1E-28	1E-30	2.6E-09 4.2E-20
ΔsucA	1 1	0.01174 5.3E-06	7.5E-07 1E-06	0.00358 0.000254	3E-05 8E-25	3E-07	0.00139 2.9E-15
ΔsucB	4.9E-08 1.8E-11	2.5E-16 2E-45	3.83E-29 3.78E-51	4.59E-30 7.96E-45	2E-37 0.0001	1E-31	2E-25
ΔtrmE	0.0579	1.2E-05 1.2E-22	2.91E-08 1.98E-33	3.2E-06 5.44E-34	3E-09 6E-49	4E-10	8.7E-05
ΔycaD	0.0013 0.14602	3E-09	2.57E-16 2.78E-21	1.8E-14	5E-22 1E-36	1E-17 6E-35	 2E-21
ΔyjbJ	0.00129	8.6E-13	3.74E-19	1.56E-18	1E-28	5E-25	 5.3E-16

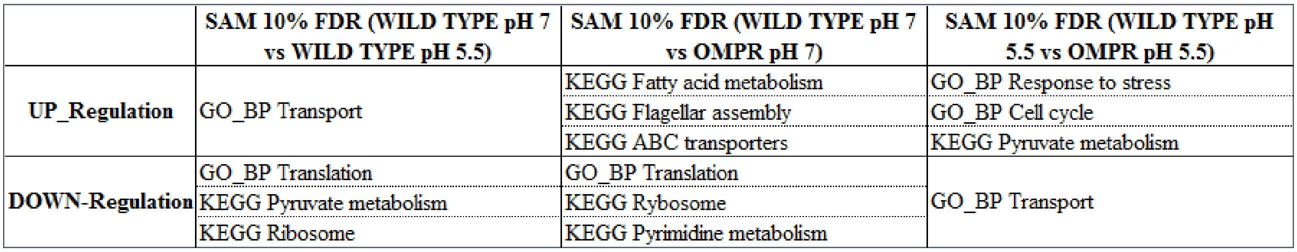
Supplementary table 2

Adapted	5' Acid	30' Acid	1 hour Acid	1 hr 30' Acid	2 hours Acid	2 hrs 30' Acid	3 hours Acid
ΔaceE	0.075344 0.083409	1.03E-11	0.0072696 8.913E-07	3.841E-05	3.9E-11 7E-16		6.54E-15 1.1E-07
ΔadiA	0.878016 1	0.042762	0.6154029 0.0915942	0.6397546	0.2548 0.16437	0.95048	0.230664 0.020962
ΔcadA	1 1.36E-07	1.74E-06 4.1E-09	0.1649808 2.863E-08	0.1018168 8.186E-07	0.7431 9.4E-08	0.18051 1.3E-05	0.006344
ΔcadB	0.17636 0.574604	1.46E-06 1.4E-09	4.116E-07 7.616E-10	1.363E-15 1.861E-10	2.6E-14 1.7E-11	7.3E-08 4.9E-11	2.82E-13
Δcrp	 0.789608	0.308973 0.20964	0.0832009 0.0118623	0.0053511 0.0009768	0.03088 4.3E-05	0.00186 6E-06	0.10117
Δfis	0.004501 0.000542	3.51E-10 4.36E-12	2.595E-14 2.313E-11	1.111E-13 2.45E-09	4.6E-05 2.6E-11	2.2E-15 7E-08	1.35E-22 6.36E-12
ΔfliA	0.546682 0.714445		0.0965822 0.8906121	0.0176328	0.00012 0.46369		1.59E-07 1.16E-06
Δfnr	0.318917 0.682717	0.000818	0.0432246 0.0331891	0.0023893	0.06904 0.0007	0.02061	0.013721 0.000123
ΔgadB	0.012873 0.362411	0.002142	0.0025326 0.0001424	0.0006941	0.12124 0.00052	0.01533	8.17E-05 0.003703
ΔgadE	1	0.077068	0.0573446	0.2011729	0.00946	0.0205	3.16E-14
ΔgadW	0.23513	0.205339	0.8103924	0.1889988	0.06232	0.16803	0.017468
ΔgadX	0.574604	0.029604	0.7726554	0.8361599	0.24237	0.04366	0.534169
Δgcd	1	3.28E-07	2.345E-10	3.43E-09	1.7E-13	0.00517	2.64E-06
ΔgcvP	 0.670637	3.87E-14 2.4E-21	9.035E-17 7.685E-18	2.127E-08 4.834E-17	2.2E-14 6.2E-24	8.4E-16	2E-18
ΔgrxA	0.057137 0.174001	1.07E-07	1.779E-12 4.047E-22	5.483E-13	5.1E-17 5.1E-35	0.0009	2.86E-12 5.05E-31
ΔhdeA	0.544708 5.48E-08	0.000328 4.98E-05	0.0159322 0.0036791	3.699E-05 0.0037073	4.4E-06 0.00053	0.00385 0.29809	7.73E-10
ΔhdeB	6.59E-06 0.107933	2.8E-06 3.28E-15	1.876E-09 8.386E-21	3.827E-09 2.338E-23	7.9E-16 2.2E-26	5.1E-10 2E-20	4.89E-11
ΔhemE	1	1.9E-09	0.0003275	7.779E-09	1.4E-14	8.8E-08	
Δhns	1 2.37E-05	0.934674 0.000203	0.7014699 1.251E-10	0.5832177 1.855E-12	0.06495 3.9E-10	0.39455 0.00478	0.609855
ΔhslJ	9.53E-05 0.001909	2.02E-22	2.709E-23 2.576E-35	1.879E-24	6.8E-24 7.7E-41	2E-18	1.25E-24 1.48E-34
ΔihfA	0.341107		0.5706497	0.0100294	0.02024		5.21E-06
ΔihfB	0.003852	3.11E-31	2.489E-38	7.414E-39	3.5E-43	6.6E-33	
ΔnarJ	0.028442 0.760854		2.514E-10 0.00127	1.331E-10	6.4E-11 0.00012		1.11E-19 2.74E-21
ΔnarX	0.001453 0.648651	2.01E-16	6.157E-17 9.894E-25	7.956E-12	2.2E-24 1.6E-31	1.5E-17	4.91E-16 7.19E-29
ΔosmC	0.661035	2.75E-07	0.0002735	2.585E-05	2.3E-06	0.00609	0.019574
ΔphoB	0.458968	0.060055	0.47284	0.0818702	0.04829	0.74503	0.006774
ΔphoH	0.042711 1.3E-07	5.15E-05 4.19E-07	0.0004196 1.359E-06	0.0001068 7.666E-05	5E-07 2E-07	0.0003	8.83E-08
ΔphoP	0.247232 0.016086	3.51E-05 1.16E-07	1.611E-06 8.401E-11	1.45E-07 7.264E-11	1.2E-10 1.4E-18	0.00012	5.85E-07
ΔphoQ	0.107349 0.029016	8.02E-05 0.000224	1.313E-07 0.0006113	7.916E-18 0.0001233	1.6E-21 0.00012	2.1E-15	5.25E-22
ΔrpoD	0.132226 0.737511	0.001913	0.009447 0.0963165	0.0015525	5.7E-05 0.00063	0.0196	3.23E-05 0.000346
ΔrpoS	0.191247 0.045437	0.005896	2.734E-08 5.821E-07	1.593E-13	2.1E-14 9.7E-07	1.7E-07	8.62E-19 7.88E-07
Δspy	0.450564 0.346179	6.61E-09	1.762E-07 4.915E-08	5.684E-12	1.4E-13 2.2E-17	7.9E-09	1.31E-11 4.17E-19
ΔsucA	0.682717 0.05026	0.000509 1.18E-07	0.0079459 2.774E-05	3.479E-05 3.005E-05	5.9E-09 3.8E-07	0.00179	1.29E-06
ΔsucB	0.182463 1.47E-10	3.52E-05 6.12E-48	3.569E-06 1.833E-43	1.45E-07 1.016E-43	0.00052 7.5E-45	1.5E-06	6.02E-09
ΔtrmE	0.260049 0.000138	0.005123 7.77E-18	0.0501222 1.809E-20	0.0300787 1.52E-25	0.00518 2.1E-28	0.12411 1.1E-22	0.002365 1.82E-21
ΔycaD	0.079489 0.004901	1.09E-15	7.44E-29 3.524E-37	2.407E-31	9.9E-35 3.8E-38	1.3E-27	1.58E-30 2.06E-36
ΔyjbJ	0.217782	1.7E-22	5.91E-30	4.08E-28	1.8E-36	6.2E-26	

Supplementary table 3

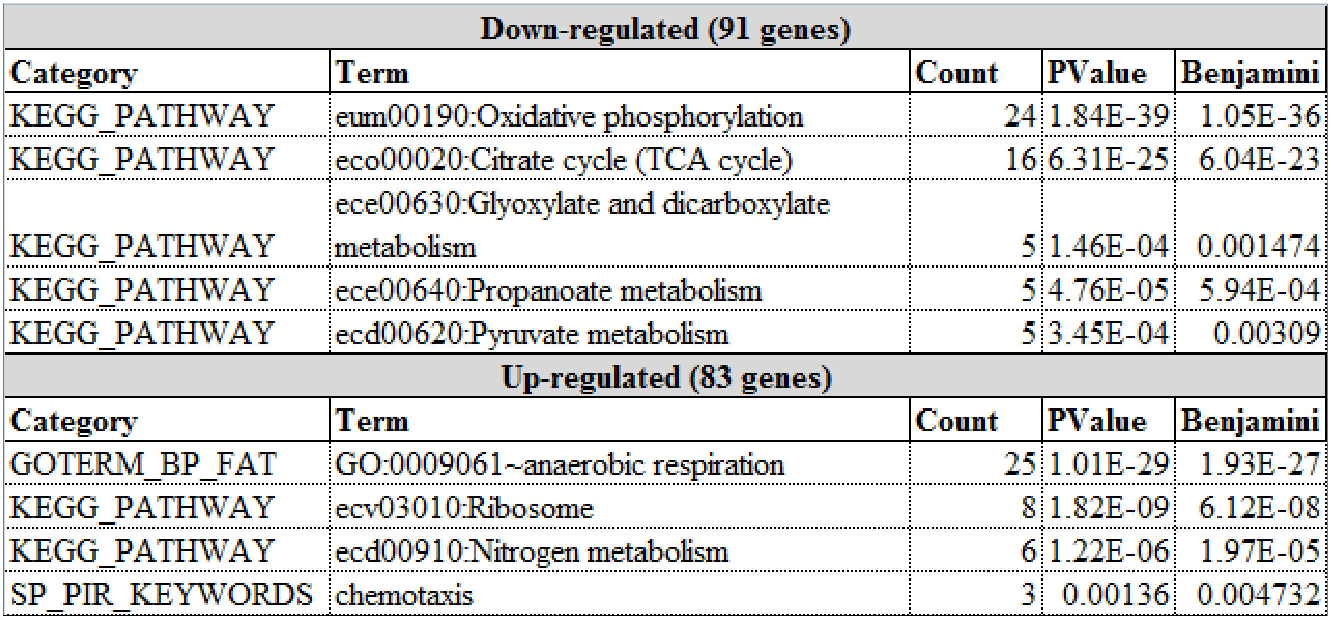
GO_BP Nitrogen compound biosynthetic process	
aroM	Protein aroM
asnC	Asparaginyl-tRNA synthetase; Regulatory protein asnC
bioF	8-amino-7-oxononanoate synthase
cysJ	Sulfite reductase [NADPH] flavoprotein alpha-component
fliY	Cystine-binding periplasmic protein
folE	GTP cyclohydrolase 1
gltI	Glutamate/aspartate periplasmic-binding protein
ilvM	Acetolactate synthase isozyme 2 small subunit
metA	Homoserine O-succinyltransferase
nadC	Nicotinate-nucleotide pyrophosphorylase [carboxylating]
ndk	Nucleoside diphosphate kinase
panD	Aspartate 1-decarboxylase
pheL	Phe operon leader peptide
pheP	Phenylalanine-specific permease
potA	Spermidine/putrescine import ATP-binding protein potA
serS	Seryl-tRNA synthetase
thiD	Phosphomethylpyrimidine kinase
yqeA	Carbamate kinase-like protein yqeA
SP_PIR Lipopolysaccharide biosynthesis	
eptB	Phosphoethanolamine transferase
lpcA	Phosphoheptose isomerase
rfaF	ADP-heptose--LPS heptosyltransferase 2
rfaJ	Lipopolysaccharide 1,2-glucosyltransferase
rfe	Undecaprenyl-phosphate alpha-N-acetylglucosaminyl 1-phosphate transferase
SP_PIR Sugar transport	
agaD	N-acetylgalactosamine permease IID component
agaV	N-acetylgalactosamine-specific phosphotransferase enzyme IIB component 2
malF	Maltose transport system permease protein malF
sgcC	Putative permease IIC component
ulaA	Ascorbate-specific permease IIC component ulaA
xylE	D-xylose-proton symporter
ycjP	Inner membrane ABC transporter permease protein ycjP
GO_BP Anaerobic respiration	
acnA	Aconitate hydratase 1
acnB	Aconitate hydratase 2
hyfC	Hydrogenase-4 component C
hyfE	Hydrogenase-4 component E
menA	1,4-dihydroxy-2-naphthoate octaprenyltransferase
nrfF	Formate-dependent nitrite reductase complex subunit nrfF
nuoF	NADH-quinone oxidoreductase subunit F
nuoN	NADH-quinone oxidoreductase subunit N
KEGG ABC Transporters	
cydC	ATP-binding/permease protein cydC
fhuD	Iron(3+)-hydroxamate-binding protein fhuD
fliY	Cystine-binding periplasmic protein
gltI	Glutamate/aspartate periplasmic-binding protein
malF	Maltose transport system permease protein malF
potA	Spermidine/putrescine import ATP-binding protein potA
GO_BP Aerobic respiration	
acnA	Aconitate hydratase 1
acnB	Aconitate hydratase 2
cyoA	Ubiquinol oxidase subunit 2
cyoB	Ubiquinol oxidase subunit 1
cyoD	Cytochrome o ubiquinol oxidase protein cyoD
nuoF	NADH-quinone oxidoreductase subunit F
nuoN	NADH-quinone oxidoreductase subunit N
ppc	Phosphoenolpyruvate carboxylase
sdhC	Succinate dehydrogenase cytochrome b556 subunit
sdhD	Succinate dehydrogenase hydrophobic membrane anchor subunit
ubiA	4-hydroxybenzoate octaprenyltransferase
KEGG Oxidative phosphorylation	
cyoA	Ubiquinol oxidase subunit 2
cyoB	Ubiquinol oxidase subunit 1
cyoD	Cytochrome o ubiquinol oxidase protein cyoD
nuoF	NADH-quinone oxidoreductase subunit F
nuoN	NADH-quinone oxidoreductase subunit N
sdhC	Succinate dehydrogenase cytochrome b556 subunit
sdhD	Succinate dehydrogenase hydrophobic membrane anchor subunit
KEGG TCA Cycle	
acnA	Aconitate hydratase 1
acnB	Aconitate hydratase 2
sdhC	Succinate dehydrogenase cytochrome b556 subunit
sdhD	Succinate dehydrogenase hydrophobic membrane anchor subunit

Supplementary table 4



	SAM 10% FDR (WILD TYPE pH 7 vs WILD TYPE pH 5.5)	SAM 10% FDR (WILD TYPE pH 7 vs OMPR pH 7)	SAM 10% FDR (WILD TYPE pH 5.5 vs OMPR pH 5.5)
UP_Regulation	GO_BP Transport	KEGG Fatty acid metabolism	GO_BP Response to stress
		KEGG Flagellar assembly	GO_BP Cell cycle
		KEGG ABC transporters	KEGG Pyruvate metabolism
DOWN-Regulation	GO_BP Translation	GO_BP Translation	GO_BP Transport
	KEGG Pyruvate metabolism	KEGG Rybosome	
	KEGG Ribosome	KEGG Pyrimidine metabolism	

Supplementary table 5



Down-regulated (91 genes)

Category	Term	Count	PValue	Benjamini
KEGG_PATHWAY	eum00190:Oxidative phosphorylation	24	1.84E-39	1.05E-36
KEGG_PATHWAY	eco00020:Citrate cycle (TCA cycle)	16	6.31E-25	6.04E-23
KEGG_PATHWAY	ece00630:Glyoxylate and dicarboxylate metabolism	5	1.46E-04	0.001474
KEGG_PATHWAY	ece00640:Propanoate metabolism	5	4.76E-05	5.94E-04
KEGG_PATHWAY	ecd00620:Pyruvate metabolism	5	3.45E-04	0.00309

Up-regulated (83 genes)

Category	Term	Count	PValue	Benjamini
GOTERM_BP_FAT	GO:0009061~anaerobic respiration	25	1.01E-29	1.93E-27
KEGG_PATHWAY	ecv03010:Ribosome	8	1.82E-09	6.12E-08
KEGG_PATHWAY	ecd00910:Nitrogen metabolism	6	1.22E-06	1.97E-05
SP_PIR_KEYWORDS	chemotaxis	3	0.00136	0.004732

Supplementary table 6

Supplementary figures and tables

Supplementary figure 1: Phenotype controls for the flow cytometry analysis

Flow cytometry analysis of living cells at pH 7 (quadrant left bottom) and dead cells, previously treated with EtOH (quadrant left up). Propidum Iodide and BOX were used as fluorescence dies on the Y and X axis respectively. These distributions were used in subsequent experiments to infer proportions of living and dead cells at various times after acidification.

Supplementary figure 2: Variability between samples at pH 7 in continuous culture experiments

The above graph shows plots of control pH 7 culture collected after reaching steady state as described in materials and methods at a) 0min, b) 15min, c)30min and d)1hour of growth at pH7 in a microarray experiment. On the X-axis the mean log signal intensity across the four time points is plotted against the log signal intensity of each replicate subtracted from the mean divided by the mean log signal intensity. The graphs clearly show that the variation between replicates is about 5%. The median of coefficient of variation between the control replicates is 10%.

Supplementary figure 3: Identification of the genes modulated during acid adaptation at operon level

We have considered the most modulated functions: S2 (A) Oxidative phosphorylation, with some of the representative enzymes; S2(B) Anaerobic respiration; S2 (C) Glycolysis/Gluconeogenesis; S2(D) ABC transporters. In the figure are shown some of the components belonging to the modulated functions and their regulation at the operonlevel. The red arrows are indicative of up-regulation, viceversa for the green arrows.

Supplementary figure 4-(1-7): Identification of the pathways modulated in response to acid adaptation

The figures represent the genes for each considered pathway, which are modulated in response to acid. In red the genes up-regulated and in green the genes down-regulated.

Supplementary figure 5: SAM analysis of the three replicates at pH 7 and pH 5.5 of the wild type strain, 15 minute time point

The SAM analysis (10% FDR) found 973 genes down-regulated and 898 genes up-regulated. After a functional annotation analysis, we have found that most of the functions down-regulated are involved in the energy metabolism and the up-regulated are characterised by genes coding for membrane transporters.

Supplementary figure 6: Gene expression profiles of selected genes in the three replicates

Expression levels of each gene are shown at pH 7 and at pH 5.5. The error bars represent standard deviations of the three replicates.

Supplementary figure 7 (1-4): Binding site analysis of potential OmpR target

The potential binding sites of the OmpRare shown in figure 1-3, which were detected in the base of the similarity with *ompC-box. For each gene considered, the genome sequence, the statistical relevance of the similarity and the relative operon are shown. Figures 7 (4-5) are related to the same analysis, but considering ompF-box. PWM scores and SEP scores were determinant for the selection of the targets.*

Supplementary figure 8: Principal component analysis of the gene KO

The three replicates of the wild type are indicated in blue for the pH 7 and in red for the pH 5.5. The mutants at pH 7 are represented by the light blue dots, at pH 5.5 by the pink dots. The first component, which defines the shift following acid exposure, is on the X axis.

Supplementary figure 9-(1-7): Identification of the pathways modulated in response to acid adaptation for the genes predictive of the phenotype outcome

The figures represent the genes for each considered pathway, which are modulated in response to acid. In red the genes up-regulated and in green the genes down-regulated.

Supplementary figure 10: Schematic representation of the genes involved in the regulation of the osmotic response

The proteins expressed on the inner membrane (IM) and periplasmic membrane (PM) are represented in the figure. The green arrows indicate the effect of down-regulation, the red arrows instead are for the up-regulation after acid adaptation.

Supplementary table 1: Selection of the genes from the wild type time course

Supplementary tables 2-3: Mutant strains showing a significant acid response phenotype

The tables show, for each mutant, the p-values obtained from the Fisher exact test. p-values were computed on the percentages of healthy cells as defined by the flow cytometry. Where two p-values are present, two biological replicates were considered. Supplementary table 2 shows the values of the not adapted mutants; in supplementary table 3 are shown the values of the adapted samples.

Supplementary table 4: Functions enriched by the genes differentially expressed between the three phenotypic groups

The analysis performed between the three groups considering log2 ratios of gene expression values gave us a list of 221 genes. We have performed a functional annotation analysis, which results are shown in this figure. We have selected the functions more represented and the representing genes.

Supplementary table 5: SAM comparison performed considering ∆*ompR at pH 7, 5.5 and the wild type*

The functions enriched by the genes differentially expressed between the wild type at pH 7 and the mutant at pH 7 (column 1), wild type pH 5.5 and mutant at pH 5.5 (column 2) and wild type at pH 7 and pH 5.5 (column 3) are represented. In the tables the functions are also represented that for each analysis were found up and down-regulated.

Supplementary table 6: FNR target genes modulation in response to acid exposure

FNR target genes were selected on the acid adaptation time course. A functional analysis was performed with DAVID, finding pathways that are up and down-regulated in response to pH 5.5, which