Orphan Enzyme		Hypothesised Gene	Prob.	Acc.	No.	Existing Annotation	Dry	Wet
1	glucosamine-6-phosphate deaminase (3.5.99.6)	YHR163W (SOL3)	$<10^{-4}$	97	8	'6-phosphogluconolactonase' ida	-	-
2	glutaminase (3.5.1.2)	YIL033C (BCY1)	$<10^{-4}$	92	11	'cAMP-dependent protein kinase inhibitor ' ida	x ?	-
3	L-threonine 3-dehydrogenase (1.1.1.103)	YDL168W (SFA1)	$<10^{-4}$	83	6	'alcohol dehydrogenase' ida	-	-
4	purine-nucleoside phosphorylase (2.4.2.1)	YLR209C (PNP1)	$<10^{-4}$	82	11	'purine-nucleoside phosphorylase' ida	✓	-
5	2-aminoadipate transaminase (2.6.1.39)	YGL202W (ARO8)	$<10^{-4}$	80	3	'aromatic-amino-acid transaminase' ida	✓	✓
6	5,10-methenyltetrahydrofolate synthetase (6.3.3.2)	YER183C (FAU1)	$<10^{-4}$	80	4	'5,10 formyltetrahydrofolate cyclo-ligase' ida	✓	-
7	glucosamine-6-phosphate deaminase (3.5.99.6)	YNR034W (SOL1)	$<10^{-4}$	79	2	'possible role in tRNA export'	-	-
8	pyridoxal kinase (2.7.1.35)	YPR121W (THI22)	$<10^{-4}$	78	1	'phosphomethylpyrimidine kinase' iss	-	-
9	mannitol-1-phosphate 5-dehydrogenase (1.1.1.17)	YNR073C	$<10^{-4}$	78	6	'putative mannitol dehydrogenase ' iss	-	-
10	1-acylglycerol-3-phosphate O-acyltransferase (2.3.1.51)	YDL052C (SLC1)	0.0001	80	6	'1-acylglycerol-3-phosphate O-acyltransferase' ida	✓	-
11	glucosamine-6-phosphate deaminase (3.5.99.6)	YGR248W (SOL4)	0.0002	78	2	'6-phosphogluconolactonase' ida	-	-
12	maleylacetoacetate isomerase (5.2.1.2)	YLL060C (GTT2)	0.0003	76	3	'glutathione S-transferase' ida	-	-
13	serine O-acetyltransferase (2.3.1.30)	YJL218W	0.0005	78	2	'unknown function'	-	-
14	L-threonine 3-dehydrogenase (1.1.1.103)	YLR070C (XYL2)	0.0052	75	6	'xylitol dehydrogenase' ida	-	-
15	2-aminoadipate transaminase (2.6.1.39)	YJL060W (BNA3)	0.0084	73	3	'kynurenine aminotransferase' ida	-	✓
16	pyridoxal kinase (2.7.1.35)	YNR027W	0.0259	76	2	'involved in bud-site selection' iss	-	-
17	polyamine oxidase (1.5.3.11)	YMR020W (FMS1)	0.0289	78	4	'polyamine oxidase' ida	✓	-
18	2-aminoadipate transaminase (2.6.1.39)	YER152C	0.0332	74	3	'uncharacterized'	-	✓
19	L-aspartate oxidase (1.4.3.16)	YJL045W	0.1300	72	1	'succinate dehydrogenase isozyme' iss	-	-
20	purine-nucleoside phosphorylase (2.4.2.1)	YLR017W (MEU1)	0.1421	72	6	'methylthioadenosine phosphorylase' ida	✓	-

Table 1

The orphan enzymes are enzymes in Adam's model of *S. cerevisiae* metabolism that did not have any genes encoding them (Oct 2006) (Whelan & King, 2008)). These orphan enzymes are included in the model because there is biochemical evidence for the associated reactions in the yeast literature. The hypothesised genes are the genes which Adam's bioinformatics-based hypothesis formation method abduced encoded the orphan enzyme.

Prob is the estimated probability of the observed accuracies at discriminating between the differences in growth curves observed with the addition of specified metabolites to the wild type and the deletant.

Acc is the highest accuracy for a metabolite species in discriminating between the growth curves observed with the addition of specified metabolites to the wild type and the deletant.

No. is the number of metabolites tested.

We carefully examined the bioinformatic databases and literature on all of the genes examined (see further information). The existing annotation shown is taken from SGD (http://www.yeastgenome.org/). (ida - inferred from Direct Assay; iss - Inferred from Sequence or structural Similarity). We particularly focussed on the ida cases.

YLR017W - Adam was correct in its hypothesis, but the experimental evidence it found was weak. N.B. PNP1/YLR209c and MEU1/YLR017w are paralogous genes that arose from a local duplication. This example puts a floor on the amount of experimental evidence required for a correct function. It is arguably an error of omission.

YLR070C – The literature evidence for xylitol dehydrogenase function is relatively weak.(Peter Richard*, Mervi H. Toivari, Merja Penttila FEBS Letters 457 (1999) 135-138)

YLL060C - Both Adam's function and the literature are plausible as there exist known genes with both functions e.g. hGSTZ1-1. (Biochem. J. (2003) *374* (731–737))

YGR248W and YHR163W are currently annotated as 6-phosphogluconolactonases. Adam's hypothesis of glucosamine-6-phosphate deaminase is closely related both chemically and enzymatically. The Nag family of proteins, which normally catalyse glucosamine-6-phosphate deaminase are absent from *S. cerevisiae,* and the closely related Sol proteins are expanded. The whole cell assays used in the literature for 6-phosphogluconolactonase do not necessarily discriminate between the two functions (Stanford, D.R., Whitney, M.L., Hurto, R.L., Eisaman, D.M., Shen, W.C., Hopper, A.K. (2004) Division of labor among the yeast sol proteins implicated in tRNA nuclear export and carbohydrate metabolism. Genetics, 168, 117-127.)

YDL168W is currently annotated as a multi-functional enzyme containing alcohol dehydrogenase and glutathione-dependent formaldehyde dehydrogenase activities, functions in formaldehyde detoxification and formation of long chain and complex alcohols. The gene is also involved in the degradation of other amino acids. Therefore, given the hydroxyl group of threonine the existing annotation does not seem contradictory to Adam's conclusion. (Richard Dickinson, R.J., Eshantha, L., SalgadoDagger, J., and Hewlins, M.J.E. (2003) The Catabolism of Amino Acids to Long Chain and Complex Alcohols in Saccharomyces cerevisiae J. Biol. Chem., Vol. 278, Issue 10, 8028-803)

YIL033C (BCY1) is predicted to be a glutaminase. This may be an error of commission by Adam. This is for the ORF YIL033C (BCY1) which Adam predicted to be a glutaminase (E.C.3.5.1.2). YIL033C is known to be a cAMP-dependent protein kinase regulatory subunit, i.e. it is not an enzyme in metabolism but a protein involved in the *control* of enzymes in metabolism. This function may be sufficient to explain the observed phenotypes. This mistake exposes a weakness in Adam's

current biological knowledge, as control of metabolism is not included. It is however possible that YIL033C is both a kinase and a glutaminase, and it is intriguing that YIL033C mutants are known to be sensitive to ammonia starvation, and that this sensitivity varies by point mutation position on YIL033C (Cannon).

Dry is a summary of the information from the manual examination of the bioinformatic databases and scientific literature. If a gene has already an associated function we do not consider this to be contradictory to Adam's conclusions unless this function is capable of explaining the observed growth phenotype, i.e. BCY1.

Wet is the result of our manual enzyme assays.