

Supplementary Table 3: Estimation of error rate for gene dispensability determination

No	Systematic ID	Gene name	Published data	Our data	Criteria for judgment	Final judgment
1	SPAC110.04c	pss1	V	E	More of CDS deleted in this study	E
2	SPAC13D6.05	alp11	E	V	Disagreement	E
3	SPAC1565.06c	spg1	E	V	Disagreement	E
4	SPAC15A10.01	atm1	V	E	More of CDS deleted in this study ¹	E
5	SPAC1B3.14	vma3	V	E	Essential in our assay conditions ²	E
6	SPAC1F7.04	rho1	E	V	Disagreement	E
7	SPAC23C11.05		V	E	More of CDS deleted in this study	E
8	SPAC2F3.03c	rpa49	V	E	More of CDS deleted in this study	E
9	SPAC30D11.13	hus5	E	V	Disagreement	E
10	SPAC343.05	vma1	V	E	Essential in our assay conditions ²	E
11	SPAC3H8.10	spo20	E	V	Disagreement	E
12	SPAC4F8.07c	hvk2	V	E	More of CDS deleted in this study	E
13	SPAC56F8.10	met9	V	E	Essential in our assay conditions ³	E
14	SPAC688.11	end4	V	E	Different interpretation due to slow growth or dying cells	E
15	SPBC14C8.16c	bot1	E	V	Disagreement	E
16	SPBC16C6.09	ogm4	V	E	Disagreement	V
17	SPBC1718.06	msp1	E	V	Disagreement	E
18	SPBC23G7.12c	rpt6	E	V	Disagreement	E
19	SPBC23G7.15c	rpp202	E	V	More of CDS deleted in this study	V
20	SPBC31E1.04	pep12	V	E	More of CDS deleted in this study	E
21	SPBC32C12.02	stel1	V	E	Disagreement	V
22	SPBC32F12.06	pch1	E	V	Disagreement	E
23	SPBC336.11		V	E	More of CDS deleted in this study	E
24	SPBC36B7.07	tlg1	V	E	Disagreement	V
25	SPBC3B9.13c	rpp102	E	V	More of CDS deleted in this study	V
26	SPBC428.19c		V	E	Published data not confirmed	E
27	SPBC725.16	res1	V	E	More of CDS deleted in this study	E
28	SPBC839.08c	its8	V	E	Different interpretation due to slow growth or dying cells	E
29	SPBC8D2.20c	sec31	E	V	Disagreement	E
30	SPCC1235.08c	pdh1	E	V	More of CDS deleted in this study	V
31	SPCC1620.11	nup97	E	V	Disagreement	E
32	SPCC1682.07	ssl1	V	E	Disagreement	V
33	SPCC1739.07		E	V	Disagreement	E
34	SPCC1739.12	ppe1	V	E	More of CDS deleted in this study	E
35	SPCC1753.04	tol1	V	E	Essential in our assay conditions ⁴	E
36	SPCC18.04	pof6	E	V	Disagreement	E
37	SPCC1840.02c	bgs4	E	V	Disagreement	E
38	SPCC306.06c		V	E	More of CDS deleted in this study	E
39	SPCC613.07		E	V	Published data not confirmed	V
40	SPCC777.10c	ubc12	E	V	Disagreement	E

E = essential and V= viable/non-essential.

¹ According to our data, the essentiality of *atm1* is dependent on the percentage of gene deleted (KO%). For example, 98% KO is E but 43% KO is V.

² The *vma1* and *vma3* genes are essential in our assay condition with medium of pH 5~6, but published as non-essential in media ~pH 7.

³ The *met9* gene is essential in our assay condition, but published as non-essential when the media is supplemented with methionine.

⁴ The *tol1* gene is essential in our assay condition, but non-essential when the media is supplemented with sulfite.

We compared the gene dispensability of 1,210 previously published deletion strains with our data, and found that 40 genes had a different dispensability to these earlier independent studies (See column "F" in **Supplementary Table 1**). For 21 of these genes, we have used our data for the assessment of dispensability for the following reasons:

- 1) **More of CDS deleted in this study (13)**: More of the CDS was deleted in our study compared with previously published work. Because removal of more of the CDS should be better to determine the deletion phenotype, we have judged that our assessment of dispensability is more likely to be correct.
- 2) **Essential in our assay conditions (4)**: The dispensability of some genes depends on specific conditions. Such genes may be essential in our assay conditions but non-essential in other specific assay conditions. We have judged our assessment of dispensability to be correct for the assay conditions we have used.
- 3) **Published data not confirmed (2)**: In the previously published work the deletion was not confirmed by sequencing. In our work we confirmed the deletion by sequencing and so judged our assessment of dispensability as more likely to be correct.
- 4) **Different interpretation due to slow growth or dying cells (2)**: Previously published work has reported a very similar phenotype with very slow growth or many cells dying, but earlier authors have interpreted this data differently to ourselves coming to differing conclusions concerning essentiality. After comparing all the data and interpretation we have judged our assessment of dispensability to be correct.
- 5) **Disagreement (19)**: Data for 19 genes were in disagreement with previously published data. We designated 15 of these genes as non-essential (previously essential) and 4 as essential (previously non-essential). Based on the worst

assumption that our data is incorrect for all of these 19 genes, the maximum error rate would be 1.6% (19/1,210). Scaling up to the entire 4,836 deletions we conclude that there could be up to 76 potential errors. For the analysis we have used the published data for these 19 genes.