

Supplemental Table 1: Yeast Strains employed in this work

Yeast Strain	Genotype	Source
BY4741	<i>MATa his3Δ1,leu2Δ0,met15Δ0,ura3Δ0</i>	
AW638	BY4741, <i>ltn1-Δ::ble^R</i>	(Letzring et al. 2013)
AW710	BY4741, <i>rpl1b-Δ::ble^R</i>	(Letzring et al. 2013)
AW714	BY4741, <i>asc1-Δ::kanMX</i>	(Letzring et al. 2013)
AW752	BY4741, <i>hel2-Δ::kanMX</i>	(Letzring et al. 2013)
AW765	BY4741,[hc, <i>URA3 ASC1</i>]	This Study
AW768	BY4741, <i>asc1-Δ::HIS5 S.p.</i> [hc, <i>URA3 ASC1</i>]	This Study
AW824	BY4741, <i>dom34-Δ::ble^R</i>	This Study
AW828	BY4741, <i>hbs1-Δ::ble^R</i>	This Study
AW850	AW765, <i>nmd2-Δ::kanMX</i>	This Study
AW854	AW765, <i>nam7-Δ::kanMX</i>	This Study
AW858	AW768, <i>nmd2-Δ::kanMX</i>	This Study
AW890	BY4741, <i>scp160-Δ::kanMX</i>	This Study
YSJ004	AW765, <i>gcn4-Δ::ble^R</i>	This Study
YSJ0012	AW768, <i>gcn4-Δ::ble^R</i>	This Study

Supplemental Table 2: Plasmids employed in this work

Plasmid Name	Description	Source
pDL202	2- μ <i>URA3 p_{PGK1}-Fluc-Rluc</i>	(Letzring et al. 2010)
pJE1012a	2- μ <i>URA3 GLN4</i> (1-187)	(Grant et al. 2012)
pEKD1024	int, <i>MET15</i> , GFP-RFP reporter, no insert upstream of GFP	(Dean and Grayhack 2012)
pAVA0577	2- μ <i>LEU2</i> vector	(Alexandrov et al. 2006)
pEAW012	int <i>P_{GAL 1,10} Rluc-GFP</i> , RFP, <i>MET15</i>	(Letzring et al. 2013)
pDL867	2- μ <i>LEU2 tR(ACG)D</i> in pAVA0577	(Letzring et al. 2010)
pDL869	2- μ <i>LEU2 tR(UCU)K-U36G</i> in pAVA0577	(Letzring et al. 2010)
pEAW0024	(CGA) ₄ insert in pEAW012	(Letzring et al. 2013)
pEAW0026	(AGA) ₄ insert in pEAW012	(Letzring et al. 2013)
pECB0498	(CGA) ₄ +1 (A)* insert in pEAW012	This Study
pECB0502	(CGA) ₄ -1 (AA)* insert in pEAW012	This Study
pECB0490	(AGA) ₄ +1 (A)* insert in pEAW012	This Study
pECB0494	(AGA) ₄ -1 (AA)* insert in pEAW012	This Study
pECB194	2- μ <i>URA3</i> vector [derived from pAVA0577]	(Alexandrov et al. 2006)
pEAW076	2- μ <i>URA3 ASC1</i> in pECB194	This Study
pEAW141	2- μ <i>LEU2 ASC1</i> No Intron in pAVA0577	This Study
pEAW0174	(AGA) ₆ insert in pEAW012	This Study
pEAW0177	(AGA) ₆ +1 (A)* insert in pEAW012	This Study
pEAW0180	(CGA) ₂ (AGA) ₄ insert in pEAW012	This Study
pEAW0183	(CGA) ₂ (AGA) ₄ +1(A)* insert in pEAW012	This Study
pEAW0186	(CGA) ₃ (AGA) ₃ insert in pEAW012	This Study
pEAW0189	(CGA) ₃ (AGA) ₃ +1(A)* insert in pEAW012	This Study
pEAW0192	(CGA) ₄ (AGA) ₂ insert in pEAW012	This Study
pEAW0195	(CGA) ₄ (AGA) ₂ +1(A)* insert in pEAW012	This Study
pEAW0198	(CGA) ₅ (AGA) insert in pEAW012	This Study
pEAW0201	(CGA) ₅ (AGA)+1(A)* insert in pEAW012	This Study
pEAW0204	(CGA) ₆ insert in pEAW012	This Study
pEAW0207	(CGA) ₆ +1(A) insert in pEAW012	This Study
pEJW003	<i>Gln4</i> (a.a.1-5)-(AGA) ₄ -GFP in EKD1024	This Study
pEJW007	<i>Gln4</i> (a.a.1-5)-(CGA) ₄ -GFP in EKD1024	This Study
pEJW020	<i>Gln4</i> (a.a.1-15)-(AGA) ₄ -GFP in EKD1024	This Study
pEJW023	<i>Gln4</i> (a.a.1-15)-(CGA) ₄ -GFP in EKD1024	This Study
pEJW027	<i>Gln4</i> (a.a.1-25)-(CGA) ₄ -GFP in EKD1024	This Study
pEJW031	<i>Gln4</i> (a.a.1-25)-(CGA) ₄ -GFP in EKD1024	This Study
pEJW035	<i>Gln4</i> (a.a.1-44)-(AGA) ₄ -GFP in EKD1024	This Study
pEJW039	<i>Gln4</i> (a.a.1-44)-(CGA) ₄ -GFP in EKD1024	This Study
pEJW043	<i>Gln4</i> (a.a.1-62)-(AGA) ₄ -GFP in EKD1024	This Study
pEJW047	<i>Gln4</i> (a.a.1-62)-(AGA) ₄ -GFP in EKD1024	This Study
pEJW051	<i>Gln4</i> (a.a.1-99)-(AGA) ₄ -GFP in EKD1024	This Study
pEJW056	<i>Gln4</i> (a.a.1-99)-(AGA) ₄ -GFP in EKD1024	This Study
pEAW234	<i>Gln4</i> (a.a.1-25)-(CGA) ₄ +1 (A)* -GFP in EKD1024	This Study
pEAW236	<i>Gln4</i> (a.a.1-25)-(AGA) ₄ +1 (A)* -GFP in EKD1024	This Study
pEAW238	<i>Gln4</i> (a.a.1-44)-(CGA) ₄ +1 (A)* -GFP in EKD1024	This Study
pEAW240	<i>Gln4</i> (a.a.1-44)-(AGA) ₄ +1 (A)* -GFP in EKD1024	This Study

pEAW242	<i>Gln4</i> (a.a. 1-62)-(CGA) ₄ +1 (A)* -GFP in EKD1024	This Study
pEAW244	<i>Gln4</i> (a.a. 1-62)-(AGA) ₄ +1 (A)* -GFP in EKD1024	This Study
pEAW246	<i>Gln4</i> (a.a. 1-99)-(CGA) ₄ +1 (A)* -GFP in EKD1024	This Study
pEAW248	<i>Gln4</i> (a.a. 1-99)-(AGA) ₄ +1 (A)* GFP in EKD1024	This Study
pEAW250	<i>Gln4</i> (a.a. 1-5)-(AGA) ₄ +1 (A)* GFP in EKD1024	This Study
pEAW252	<i>Gln4</i> (a.a. 1-5)-(CGA) ₄ +1 (A)* GFP in EKD1024	This Study
pEAW254	<i>Gln4</i> (a.a. 1-15)-(AGA) ₄ +1 (A)* GFP in EKD1024	This Study
pEAW256	<i>Gln4</i> (a.a. 1-15)-(CGA) ₄ +1 (A)* GFP in EKD1024	This Study
pEAW264	(CGA) ₃ +1 (A) insert in pEAW012	This Study
pEAW270	(CGA) ₂ AGA +1 (A) insert in pEAW012	This Study
pEAW275	(AGA) ₃ insert in pEAW012	This Study
pEAW276	(AGA) ₃ +1 (A) insert in pEAW012	This Study
pEAW277	(CGA) ₃ insert in pEAW012	This Study
pEAW282	(CGA) ₂ AGA insert in pEAW012	This Study
pEAW0320	(AGA) ₄ insert in pDL202	This Study
pEAW0322	(CGA) ₄ insert in pDL202	This Study
pEAW0324	(CGA) ₄ +1 (A) insert in pDL202	This Study
pEAW0326	(AGA) ₄ +1 (A) insert in pDL202	This Study

*Indicates the nucleotide(s) that were inserted directly after Arg codons to put GFP into either the +1 or -1 frame

Supplemental Table S3: Oligonucleotides Employed in this work

Oligo ID	Description	Sequence (5'-3')
OW125	ASC1 delta phleo F (PCR deletion cassette)	ATAACACACTAAAGTAAATAAAGTGAAAAGCGGCCGCCAGCTGAA
OW126	ASC1phelo Reverse (PCR deletion cassette)	CATAAAAGAACAAATGAACTTTATACATATTCAAATAGGCCACTAGTGGATCTGAT
OW016	ASC1-482F	CACGTATTTTGCTCGGTGTGATAT
OW017	ASC1-339R	GAGACTGAATTTAATGAACTCCTTGC
OW070	ASC1-326+LIC F (Clone ASC1)	GGGTCCTGGTTCGAGCCATCTGTAGC
OW071	ASC1+434+LIC R (Clone ASC1)	CTTGTTCTGTGCTGTTACTCTATCTAC
OW090	RLUC-GFP Fusion F	CTAATTCATCAACCTTACGAGCAAGGATCTATGACTTCG
OW091	RLUC-GFP Fusion R	TACCACTATTAATTAAGTTACTAGAGGTGACATTTGTTTCATTTTTGAGAACTC
OW119	ASC1-496P1 F (Clone ASC1 No Intron)	GCATTATGAATTGGAACCACGTAT
OW120	ASC1-482+LIC (Clone ASC1 No Intron)	GGGTCCTGGTTCGAGCATTATGAATTGGAACCACGTAT
OW121	ASC1+537 Intron R (Clone ASC1 No Intron)	CCTTAACCATTTTGTCGTTACCG
OW122	ASC1+810intron P3 F (Clone ASC1 No Intron)	AAAATGGTTAAGGCTTGGAACTTAAACCAATTC
OW123	Asc1+445 P4 R (Clone ASC1 No Intron)	CAATTGCTCTATCTACCTATGGAATG
OW124	ASC1 +445 P4 R+Lic (Clone ASC1 No Intron)	CTTGTTCTGTGCTGTTACAATTGCTCTATCTACCTATGGAATG
OJYW001	GLN4(a.a.1-5)(AGA)4-GFP F	AATTCATCAACCTTAAGAAAATGTCTTCTGTAGAAAGAAGAAGAAGA
OJYW002	GLN4(a.a.1-5)(AGA)4-GFP R	CTTCCAAACCACTGGTTCCTTCTTCTTCTTCTACAGAAGACAT
OJYW003	GLN4(a.a.1-5)(CGA)4-GFP F	AATTCATCAACCTTAAGAAAATGTCTTCTGTAGAACGACGACG
OJYW004	GLN4(a.a.1-5)(CGA)4-GFP R	CTTCCAAACCACTGGTTCGTCGTCGTCGTTCTACAGAAGACAT
OJYW008	GLN4 (a.a.1-16) F	AATTCATCAACCTTAAGAAAATGTCTTCTGTAGAAAGAATTGACTCAGCTGTTTTACAG
OJYW009	GLN4(a.a.1-16) (AGA)4 R	CTTCCAAACCACTGGTTCCTTCTTCTTCTCCCAACCTGTGAAAACAGCTGAGTCAATTCTT
OJYW010	GLN4(a.a.1-16) (CGA)4 R	CTTCCAAACCACTGGTTCGTCGTCGTCGCCAACCTGTGAAAACAGCTGAGTCAATTCTT
OJYW011	GLN4 (a.a. 1-(25, 44, 62, 99)) F	AATTCATCAACCTTAAGAAAATGTCTTCTGTAGAAAGAATTGACTCAG
OJYW012	GLN4(a.a.1-25) (AGA)4 R	CTTCCAAACCACTGGTTCCTTCTTCTTCTGACAATCTCCTTCACCTTTTTATCTTC
OJYW013	GLN4(a.a.1-25) (CGA)4 R	CTTCCAAACCACTGGTTCGTCGTCGTCGGACAATCTCCTTCACCTTTTTATCTTC
OJYW014	GLN4(a.a.1-44) (AGA)4 R	CTTCCAAACCACTGGTTCCTTCTTCTTCTGTCCGAAGGAGTCTCCTTAATCA
OJYW015	GLN4(a.a.1-44) (CGA)4 R	CTTCCAAACCACTGGTTCGTCGTCGTCGGTCCGAAGGAGTCTCCTTAATCA
OJYW016	GLN4(a.a.1-62) (AGA)4 R	CTTCCAAACCACTGGTTCCTTCTTCTTCTGACAAAAGATGCCAGGTTGTGTA
OJYW017	GLN4(a.a.1-62) (CGA)4 R	CTTCCAAACCACTGGTTCGTCGTCGTCGGACAAAAGATGCCAGGTTGTGTA
OJYW018	GLN4(a.a.1-99) (AGA)4 R	CTTCCAAACCACTGGTTCCTTCTTCTTCTGCCATTAGCCTTTACATATTTAAATGC
OJYW019	GLN4(a.a.1-99) (CGA)4 R	CTTCCAAACCACTGGTTCGTCGTCGTCGGCCATTAGCCTTTACATATTTAAATGC
OW206	GLN4 (a.a.1-5)(AGA)4+1-GFP F	AATTCATCAACCTTAAGAAAATGTCTTCTGTAGAAAGAAGAAGAAGAA
OW207	GLN4 (a.a.1-5)(AGA)4+1-GFP R	CTTCCAAACCACTGGTTCCTTCTTCTTCTTCTACAGAAGACAT

Oligo ID	Description	Sequence (5'-3')
OW191	RLUC- (CGA)2(AGA)4+1-GFP [R]	CTTCCAAACCACTTTCTTCTTCTCT
OW192	RLUC- (CGA)3(AGA)3-GFP [R]	CTTCCAAACCACTTCTTCTTCTCG
OW193	RLUC-(CGA)3(AGA)3 +1-GFP [R]	CTTCCAAACCACTTTCTTCTTCTCG
OW198	RLUC-(CGA)5(AGA)-GFP [R]	CTTCCAAACCACTTCTTCGTCTGTCG
OW199	RLUC-(CGA)5(AGA) +1-GFP [R]	CTTCCAAACCACTTTCTTCGTCTGTCG
OW200	RLUC- (AGA)6-GFP [R]	CTTCCAAACCACTTCTTCTTCTTCT
OW201	RLUC- (AGA)6 +1-GFP [R]	CTTCCAAACCACTTTCTTCTTCTTCT
OW202	RLUC-(CGA)4(AGA)2-GFP [R]	CTTCCAAACCACTTCTTCTTCTGTCG
OW203	RLUC-(CGA)4(AGA)2+1-GFP [R]	CTTCCAAACCACTTTCTTCTTCTGTCG
OW204	RLUC- (CGA)6-GFP [R]	CTTCCAAACCACTTCGTCTGTCGTCG
OW205	RLUC-(CGA)6+1-GFP [R]	CTTCCAAACCACTTTCTGTCGTCTGTCG
OCB087	PCR DOM34 deletion F	GTACTGCGTGATGAAAGTGATACATACATTATTCAATATTCCAGCTGAAGCTTCGTACGC
OCB088	PCR DOM34 deletion R	TAATTGCGGCTTCACATCTACTTGCTTATAGGGAAGAAGTGCATAGGCCACTAGTGGATC
OCB089	Extension PCR DOM34 deletion F	AGCAGAGCTGAAAAAATTTTCTTTTTACCAAAAATCCGTAAGTGCATGAAAGTGTAC
OCB090	Extension PCR DOM34 deletion R	ACGTGACTAACTAGAACCATTTTACAACAACCTCAATTAATTGCGGCTTCACATCTACTTG
OCB091	PCR HBS1 deletion F	GCCCATTAATGTGACTTGAATATACCACATGAAAGCTAACCAGCTGAAGCTTCGTACGC
OCB092	PCR HBS1 deletion R	CAGAGGCAGGTATTCTTTTGTATTGTGCGCCCTTAACCTTGCATAGGCCACTAGTGGATC
OCB093	Extension PCR HBS1 deletion F	TCGTATTCATATATTGATCACAATGCACGGAGACGCCATTAATGTGACTTGAATATACC
OCB094	Extension PCR HBS1 deletion R	TTTTCAAACCTATTCAATAGTTTTTTAGATATATTCAGAGGCAGGTATTCTTTTGTATTG
OW0162	SCP160-276 F (PCR deletion)	GACTTGCAACGGATAGTATATTTAC
OW0163	SCP160-357 F (confirm deletion)	GTGGGCTTGAAAAATTTTCGCTC
OW0164	SCP160 +182 R (PCR deletion)	CTTCTCCATTTCTTATGTTTCGTG
OW0242	RLUC- (AGA)3+1-GFP [F]	ACCTCTACTAACTTAAGAAGAAGAAA
OW0259	RLUC- (AGA)3+1-GFP [R]	CTTCCAAACCACTTTCTTCTTCT
OW0260	RLUC- (AGA)3-GFP [R]	CTTCCAAACCACTTCTTCTTCT
OW0261	RLUC- (AGA)6-GFP [F]	ACCTCTACTAACTTAAGAAGAAGAA
OW0270	RLUC- (CGA)2AGA+1-GFP [R]	CTTCCAAACCACTTTCTTCGTCTG
OW0271	RLUC- (CGA)2AGA+1-GFP [F]	ACCTCTACTAACTTACGACGAAGAAA
OW0248	RLUC- (CGA)2AGA -GFP [F]	ACCTCTACTAACTTACGACGAAGAA
OW0249	RLUC- (CGA)2AGA-GFP [R]	CTTCCAAACCACTTCTTCGTCTG
OW0257	RLUC- (CGA)3 -GFP [R]	CTTCCAAACCACTTCGTCTGTCG
OW0258	RLUC- (CGA)3-GFP [F]	ACCTCTACTAACTTACGACGACGAA
OW0282	RLUC- (CGA)3+1 -GFP [R]	CTTCCAAACCACTTTCTGTCGTCTG

Oligo ID	Description	Sequence (5'-3')
OW0283	RLUC- (CGA)3+1-GFP [F]	ACCTCTACTAACTTACGACGACGAAA
OW0165	SCP160 +272 R (confirm deletion)	CCGCCTTATAACGAAGACTCTTT
OW290	Nam7- 446 F (confirm deletion)	AAACATATGCATCTGGCGAACTC
OW291	Nam7-271 F (PCR deletion)	CATCAGTTTTCCCTTTGCTTACTT
OW292	Nam7+ 277 R (PCR deletion)	GCGCCGTAAAGAGTATTCATTAG
OW293	NAM7+ 324 R (confirm deletion)	GCAAAAATGAAACCTCGCTAC
OW294	Nmd2-321 F (confirm deletion)	GTTTCTATGATCACTACGGGATA
OW295	Nmd2-264 F (PCR deletion)	CTTAGGGCATGAGGATGATATTAG
OW296	Nmd2+206 R (PCR deletion)	AAACTACAGCGGTGGTAAAGAAG
OW297	Nmd2+278 R (confirm deletion)	TGTTGTCAGTTTAAGCAAGGACC
OW320	RLUC-(CGA)4-FLUC F	TCGACGCGACGACGACGAG
OW321	RLUC-(CGA)4-FLUC R	GATCCTCGTCGTCGTCGCG
OW322	RLUC-(CGA)4+1 (A) -FLUC F	TCGACGCGACGACGACGAAG
OW323	RLUC-(CGA)4+1 (A) -FLUC R	GATCCTTCGTCGTCGTCGCG
OW326	RLUC-(AGA)4-FLUC F	TCGACGAGAAGAAGAAGAG
OW327	RLUC-(AGA)4-FLUC R	GATCCTCTTCTTCTTCTCG
OW328	RLUC-(AGA)4+1 (A) -FLUC F	TCGACGAGAAGAAGAAGAAG
OW329	RLUC-(AGA)4+1 (A) -FLUC R	GATCCTTCTTCTTCTTCTCG
OCB721	RT-QPCR GFP Internal F	TATACATCACGGCAGACAAACA
OCB722	RT-QPCR GFP Internal R	GCTAGTTGAACGGAACCATCT
OACT1-F	Actin Primer F	ACG TTC CAG CCT TCT ACG TTT CCA
OACT1-R	ACT 1 Primer R	ACG TGA GTA ACA CCA TCA CCG GAA