Supplementary Figures and Tables

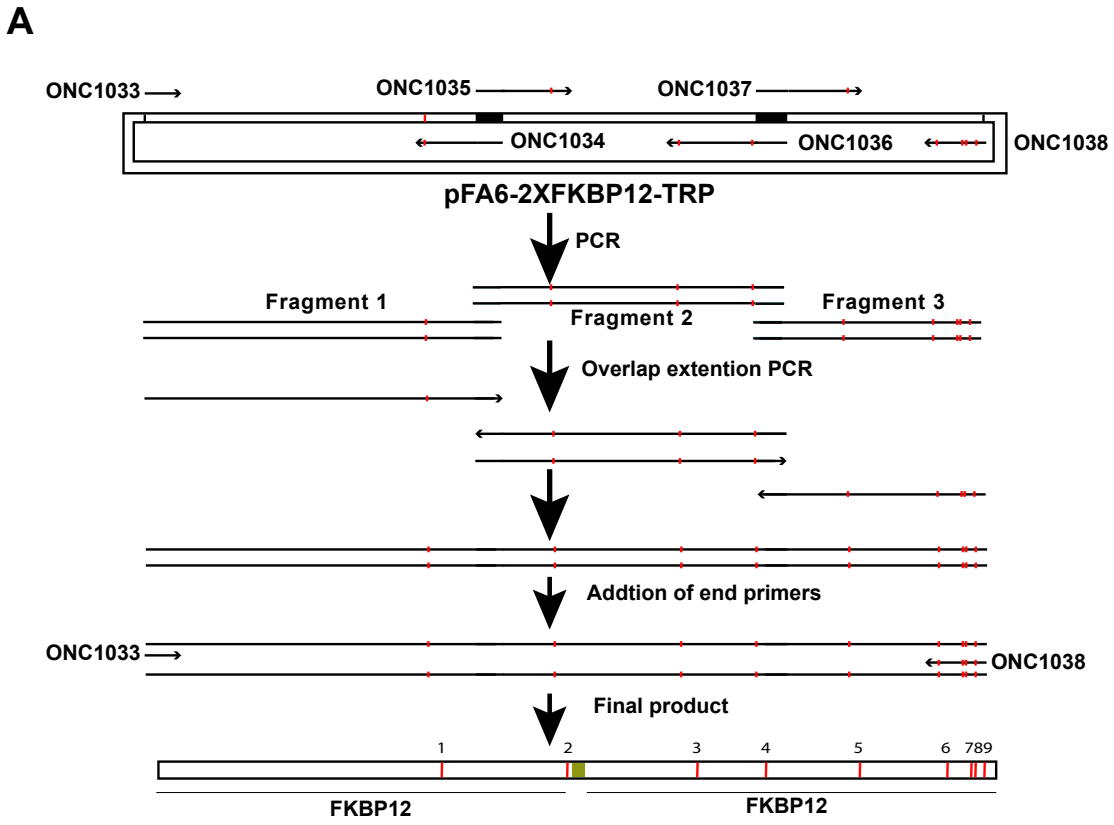
AMERICAN SOCIETY FOR MICROBIOLOGY | mSphere

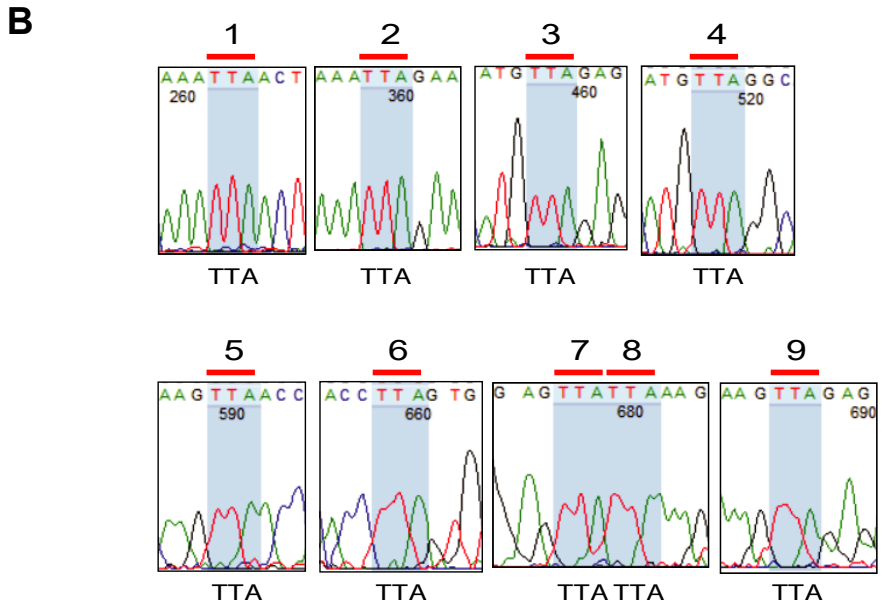
| Genetics and Molecular Biology | Resource Report

A CUG codon-adapted anchor-away toolkit for functional analysis of genes in *Candida albicans*

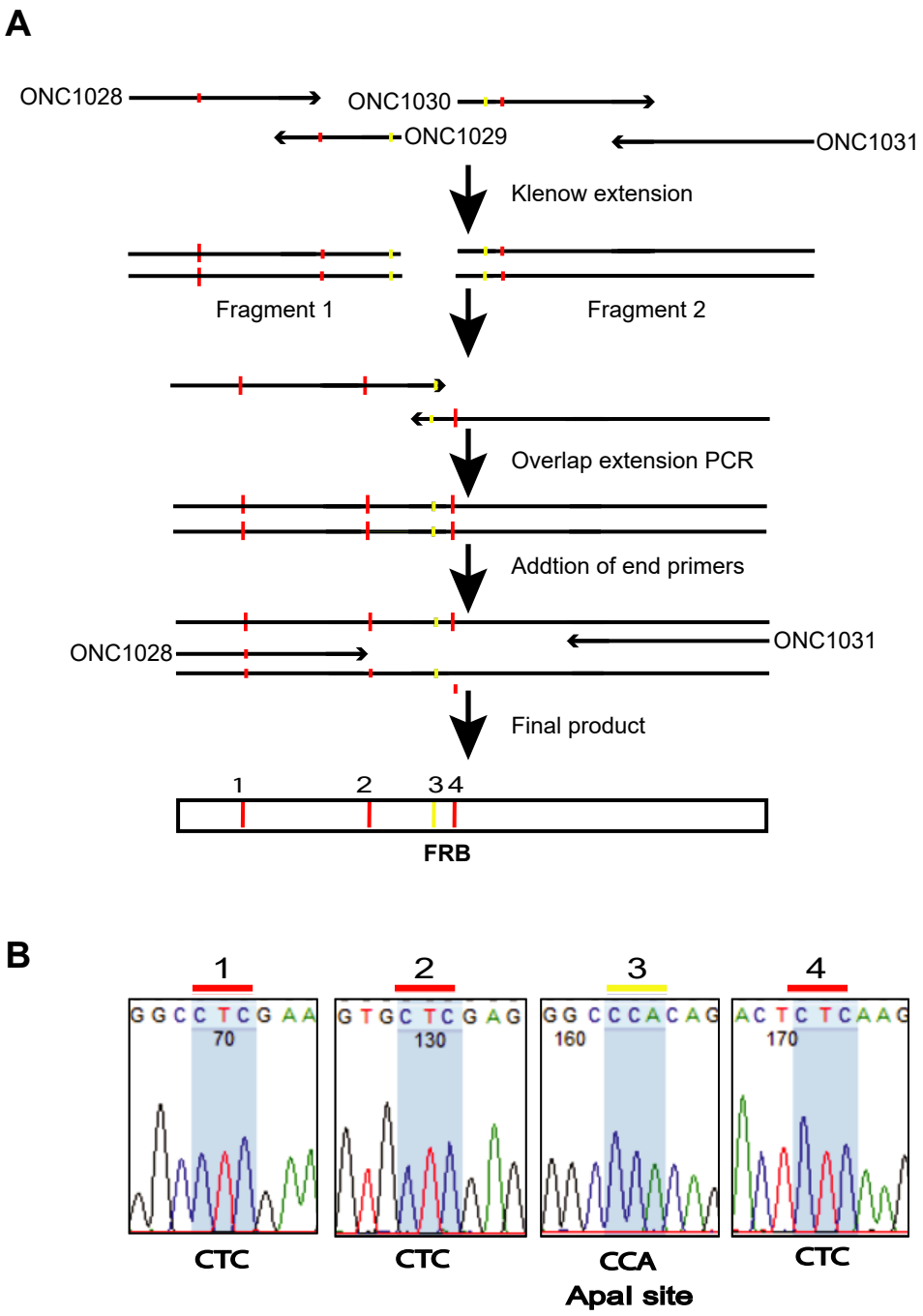
Basharat Bashir Teli,[1] Priyanka Nagar,[1] Yumnam Priyadarshini,[1] Poonam Poonia,[1] Krishnamurthy Natarajan[1]

Supplementary Figures

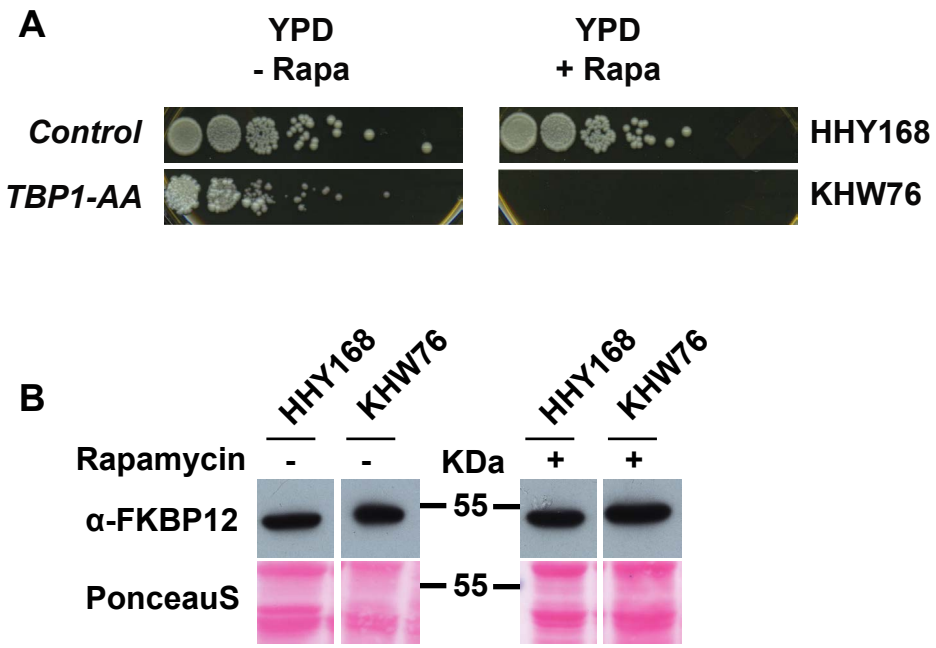




Teli et al., Fig. S1



Teli et al., Fig. S2



Teli et al., Fig. S3

SUPPLEMENTARY MATERIAL

Legends to supplementary figures:

Fig. S1. *FKBP12* CUG codon mutagenesis and confirmation. (A) Schematic diagram showing the construction of CUG-codon adapted *2xFKBP12* by overlap extension PCR mediated site-directed mutagenesis. (B) DNA sequence of the plasmid pYPC36 showing the relevant *2xFKBP* regions with codon changes from CTG to TTA.

Fig. S2. *FRB* CUG codon mutagenesis and confirmation. (A) Schematic diagram showing the construction of CUG-codon adapted *FRB* sequence by site-directed mutagenesis using Klenow extension and overlap extension PCR. (B) DNA sequence of the plasmid pBB8 showing the relevant *FRB* regions with codon changes from CTG to TTA.

Fig. S3. *S. cerevisiae TBP-AA* analysis. (A) The WT parental strain HHY168 and TBP-AA strain KHW76 were grown, spotted onto -Rapa or +Rapa YPD plates, grown for 48h and phenotype tested. (B) Expression of fusion protein TBP-FRB by Western blotting using anti-FKBP12 antibody.

Supplementary Information

Teli et al. A CUG codon-adapted anchor-away toolkit for functional analysis of genes in *Candida albicans*

Table S1. List of Strains

Strain	Relevant Genotype	Comment	Source
C.albicans Strains			
SN152	*arg4Δ/arg4Δ leu2Δ /leu2Δ his1Δ/his1Δ URA3/ura3Δ::imm434 IRO1/iro1Δ::imm434*	Wild-type strain	(1)
SN95	*arg4Δ/arg4Δ his1Δ/his1Δ URA3/ura3Δ::imm434 IRO1/iro1Δ::imm434*	Wild-type strain	(1)
SDC71	SN95 *spt8Δ::HAH1/spt8Δ::HIS1*	*spt8Δ* deletion mutant	This work
PSC1	SN152 *TOR1-1/TOR1*	*TOR1-1* Rapamycin-resistant	This work
PSC2	SN152 *TOR1-2/TOR1*	*TOR1-2* Rapamycin-resistant	This work
BB13	SN152 *RBP1/rbp1Δ : : SAT1-FLP*	*rbp1Δ*heterozygous mutant	This work
BB10	PSC2 *RBP1/rbp1Δ : : SAT1-FLP*	*rbp1Δ*heterozygous mutant	This work
BB21	PSC2 *RBP1/rbp1Δ : : FRT*	*rbp1Δ*heterozygous mutant	This work
BB29	PSC2*rbp1Δ : : FRT/rbp1Δ : : SAT1-FLP*	*rbp1Δ*homozygous mutant	This work
BB37	PSC2*rbp1Δ : : FRT/rbp1Δ : : FRT*	*rbp1Δ*homozygous mutant	This work
BB85	BB37 *RPL13/RPL13*-Primer binding site *-2XFKBP12-SAT1-FLP*	Anchor Away parental base strain	This work
BB107	BB85 *RPL13/RPL13-2XFKBP12-SAT1-FLP TBP1/TBP1 Δ : :LEU2*		This work
BB114	BB85 *RPL13/RPL13-2XFKBP12-SAT1-FLP SPT81/spt8 Δ : :LEU2*		This work
BB117	BB37 *RPL13/RPL13-2XFKBP12-FRT*		This work
BB119	BB85 *RPL13/RPL13-2XFKBP12-FRT TBP1/tbp1 Δ : :LEU2*		This work
BB120	BB85 *RPL13/RPL13-2XFKBP12-FRT SPT81/spt8 Δ : :LEU2*		This work
BB122	BB114 *SPT8-FRB-ARG4/spt8 Δ : :LEU2*		This work
BB123	BB119 *TBP1-FRB-ARG4/tbp1 Δ : :LEU2*		This work
JRB12	SC5314 *TOR1-1/TOR1*		(2)

S.cerevisiae Strains			
HHY168	*MATα, TOR1-1, fpr1::NAT, RPL13A-2xFKB12::TRP1*	Anchor Away parental base strain	(3)
KHW76	*MATα, TOR1-1, fpr1::NAT, RPL13A-2xFKB12::TRP1, SPT15-FRB::KANMX*	ScTBP(Spt15) anchor-away strain	(4)

Table S2. List of Plasmids

PLASMID	RELEVANT DESCRIPTION	SOURCE
pSN40	*C.m.LEU2,* Kan^R	(1)
pSN52	*C.d. HIS1*,Kan^R	(1)
pSN69	*C.d. ARG4*,Kan^R	(1)
p30582	pFA6a-2×FKBP12-TRP1	(3)
P30579	pFA6a-FRB-HisMX6	(3)
pLIT-SAT1/ Ip27	*SAT1FLP* cassette in pLITMUS28	(5)
pYPC36	2xFKBP12 with optimized CTG codons in the vector pLIT-*SAT1*	This work
pBB24	*ACT1t* at 3' end of 2xFKBP12 (withoptimized CTG codons) in pYPC36	This work
pBB2	FRB with optimizedCTG codons in the vector pSN52	This work
pBB8	FRB with optimized CTG codons in the vector pSN69	This work
pBB9	Second FRB with optimized CTG codons plus TAA inserted at the 5' end of first *FRB* in pBB2	This work
pBB28	*ACT1t* terminator replaced the first FRB in pBB9	This work
pBB30	FRB (optimized CTG) +TAA and *ACT1t* replacing FRB (Minus TAA) in pBB8	This work
pBB43	Spacer sequence from pFA6 series plasmid and 2xFKBP12 in place of 2xFKBP12 fragment in pBB24	This work
pBB50-1	Spacer sequence from pFA6 series plasmid and FRB and stop codon in place of FRB fragment in pBB28	This work

Table S3. List of Oligonucleotides

Oligo	Length	SEQUENCE (5' to 3')	NOTES
ONC33	20	GATATCATTTCCGATGAAGC	Universal Primer designed to test for right clones obtained after transforming LEU-based cassette.
ONC140	24	TCTTGGTGAGAACAGCGACCGAAA	Reverse primer for upstream split marker of *SAT1* flipper
ONC141	24	GGAGCGATAAGCGTGCTTCTGCCG	Forward primer for a downstream split marker of *SAT1* flipper
ONC980	82	TTTATGGCACGAACAATGGCACGATGCTTTGGAA GATGCTAGCAGGTTTTTCTTTGGTGAACACAACA CAGAAAAGATGTTT	Wild type TOR1 DNA sequence (5910 bp-5991 bp)
ONC981	82	TTTATGGCACGAACAATGGCACGATGCTTTGGAA GATGCTCGCAGGTTTTTCTTTGGTGAACACAACA CAGAAAAGATGTTT	Long oligo for point mutation in FRB domain of *TOR1* for disruption of Nhe1 site, *TOR1* to *tor1-1* (A-C)
ONC982	82	TTTATGGCACGAACAATGGCACGATGCTTTGGAA GATGCTATCAGGTTTTTCTTTGGTGAACACAACA CAGAAAAGATGTTT	Long oligo for point mutation in FRB domain of *TOR1* for disruption of Nhe1 site, *TOR1* to *tor1-2* (G-T)
ONC983	23	GAAGAATACACTAAATTGTTGGC	Forward primer to confirm *tor1-1* and *tor1-2* NheI site disruption (Position 5113 bp to 5135 bp with respect to start codon)
ONC984	21	TGGAAAGTGTCACTATTTGGA	Reverse primer to confirm *tor1-1* and *tor1-2* NheI site disruption (Position 6567 bp to 6587 bpwrt to start codon)
ONC1000	23	TAAATTCGGGCTATTGATGGTGG	Forward primer for screening present 229 bp upstream of the start codon of *RBP1*
ONC1001	21	TGTCTGAAGAACTTCCACAAA	Forward gene-specific primer starting from second base pair of start codon of *RBP1*
ONC1002	21	CACCAAGTAATTCAACTTCGA	Reverse gene-specific primer present 11 bp upstream of stop codon *RBP1*

ONC1122	22	CACAAGATCAACCACCACTAAG	Forward primer for the amplification of up split of *SAT1* flipper cassette from *RBP1Δ/rbp1Δ::SAT1-FLP* and present 486 bp upstream of the start codon of *RBP1*
ONC1123	21	TGATTCAACCTCCATTCCCCG	Reverse primer for the amplification of down split of *SAT1* flipper cassette from *RBP1Δ/rbp1Δ::SAT1-FLP* and present 719 bp downstream of the start codon of *RBP1*
ONC1124	20	AGTAGTGACTCCTGCTGCTG	Forward primer for screening present 860 bp upstream of the start codon of *RBP1*
ONC1125	21	CAAGAACAAGAGCAAGATGC	Reverse primer for screening present 871 bp downstream of start codon of *RBP1*
ONC1026	80	ATTGACACATATAACTATTATTTGCAATTATTTCATTAACTTAAATTAGATTAAATTACAGAAGTTCCTATACTTTCTAG	Forward long primer for knock out the construction of *RBP1* by *SAT1-FLP* cassette present 1 bp upstream of the start codon of *RBP1*
ONC1027	80	ACTGGGAATTTATATTACAAACCAAAATAACTAAACTAATAATTCTCTTTGTGTTTATACGAAGTTCCTATTCTCTAGAA	Reverse long primer for knock out the construction of *RBP1* by *SAT1-FLP* cassette present 54bp downstream stop codon of *RBP1*
ONC1028	91	CATCCTCTGGCATGAGATGTGGCATGAAGGCCTCGAAGAGGCATCTCGTTTGTACTTTGGGGAAAGGAACGTGAAAGGCATGTTTGAGGTG	Forward primer for Klenow extension of *FRB* fragment 1 and end primer for overlap extension PCR for *FRB* CTG codon optimisation
ONC1029	59	CTGTGGGCCCCGTTCCATCATAGCATGCAAGGGCTCGAGCACCTCAAACATGCCTTTCA	Reverse primer for Klenow extension of *FRB* fragment 1 and for *FRB* CTG codon optimization
ONC1030	90	ACGGGGCCCACAGACTCTCAAGGAAACATCCTTTAATCAGGCCTATGGTCGAGATTTAATGGAGGCCCAAGAGTGGTGCAGGAAGTACAT	Forward primer for Klenow extension of *FRB* fragment 2and for *FRB* CTG codon optimization
ONC1031	94	CTTTGAGATTCGTCGGAACACATGATAATAGAGGTCCCAGGCTTGGAGGAGGTCCTTGACATTCCCTGATTTCATGTACTTCCTGCACCACTCT	Reverse primer for Klenow extension of *FRB* fragment 2 and end primer for overlap extension PCR for *FRB* CTG codon optimisation
ONC1033	31	CGGAGTGCAGGTGGAAACCATCTCCCCAGGA	Forward primer for PCR amplification of *2xFKBP12*fragment 1 and end primer for overlap extension PCR for *2xFKBP12* CTG codon optimisation

ONC1034	73	TGGTGGGATGATGCCTGGGTGCCCAGTGGCACCATAGGCATAATCTGGAGATATAGTTAATTTGGCTCTCTGA	Reverse primer for PCR amplification of *2xFKBP12*fragment 1 for *2xFKBP12* CTG codon optimisation
ONC1035	73	ACCCAGGCATCATCCCACCACATGCCACTCTCGTCTTCGATGTGGAGCTTCTAAAATTAGAAACTAGAGGCGT	Forward primer for PCR amplification of *2xFKBP12* fragment 2 for *2xFKBP12* CTG codon optimisation
ONC1036	93	GCGGATCACTTCCTGTTTGCCTAACATGAACTTGAAGGGCTTGTTGCGGTCGCGGCTGCTGTCGAACTTCTTGCCGTCCTCTAACATGCCGGT	Reverse primer for PCR amplification of *2xFKBP12*fragment 2 for *2xFKBP12* CTG codon optimisation
ONC1037	81	GGCAAACAGGAAGTGATCCGCGGCTGGGAGGAAGGCGTGGCTCAGATGAGCGTGGGGCAGCGGGCCAAGTTAACCATCAGC	Forward for PCR amplification of *2xFKBP12* fragment 3 for *2xFKBP12* CTG codon optimisation
ONC1038	52	CTTAAGTCTCTAACTTTAATAACTCCACGTCGAACACTAAGGTGGCGTGGGG	Reverse primer for PCR amplification of *2xFKBP12*fragment 3 and end primer overlap extension PCR for *2xFKBP12* CTG codon optimisation
ONC1129	77	GGTATCAGAGAAAAGAGAGCTAAAGAAAAGGCTGAAGCCGAAGCTGAAAAAGCTAAAAAAGgagtgcaggtggaaac	Forward Primer for *RPL13-2XFKBP12* tagging
ONC1131	20	GGATGCTTGAAGATGGAAAG	Forward primer present within 2XFKBP12 tag (Position: 76bp downstream of the first codon of 2XFKBP12 tag in pYPC36, pBB24 and pBB43 plasmids)
ONC1132	20	CGGTTCAAGGTCCAATCAAT	Reverse primer for screening present (Position: 479 downstream of the stop codon of *RPL13*)
ONC1133	80	AAAAAGGTTTCAAGTATTATACATCTCCTAAACAACAACTCACTTTAATGCCAAGTTTGCGAAGTTCCTATTCTCTAGAA	Reverse Primer for *RPL13-2XFKBP12* tagging
ONC1134	20	CCAAAATACGCCAGAACCAT	Reverse primer for screening 255 bp downstream of the start codon of *RPL13*
ONC1135	28	AAAGGGCCCGAGTGAAATTCTGGAAATC	Forward primer with ApaI site and 3bp overhangs to clone *ACT1t* to pYPC36
ONC1136	29	TATGGGCCCATTTTATGATGGAATGAATG	Reverse primer with ApaI site and 3bp overhangs to clone *ACT1t* to Pypc36

ONC1137	30	AAAAAGCTTATCCTCTGGCATGAGATGTGG	Forward primer with HindIII site and 3bp overhangs to clone FRB-TAA to pBB2
ONC1138	36	ATAGAGCTCTTACTTTGAGATTCGTCGGAACACATG	Reverse primer with SacI site and 3bp overhangs to clone FRB-TAA to pBB2
ONC1139	28	AAAGAGCTCGAGTGAAATTCTGGAAATC	Forward primer with SacI site and 3bp overhangs to clone *ACT1t* to pBB9
ONC1140	30	TATGGATCCATTTTATGATGGAATGAATGG	Reverse primer with BamHI site and 3bp overhangs to clone *ACt1t* to pBB9
ONC1141	30	TATGGATCCATCCTCTGGCATGAGATGTGG	Forward primer with BamHI site and 3bp overhangs to clone FRB-TAA-*ACT1t* to pBB8
ONC1142	30	AAAACTAGTATTTTATGATGGAATGAATGG	Reverse primer with SpeI site and 3bp overhangs to clone FRB-TAA-*ACT1t* to pBB8.
ONC1147	22	GTTAACCATCAGCCCCGACTAT	Sequencing primer for addition of *ACT1t* at 3' end of 2XFKBP12
ONC1148	81	GAAGAAATTTATGATGCATTTGAACTGATTTATCCGGTTTTAAATGAATTTCGTAAAAATATCCTCTGGCATGAGATGTGG	Forward Primer for *TBP1-FRB* tagging
ONC1149	81	TTTTGAATTTTTTATAGATATCTTAAACACTTACACATATACATATGGCTTGTGTTGAAAGCCAGTGTGATGGATATCTGC	Reverse Primer for deletion*TBP1* and *TBP1-FRB* tagging
ONC1150	20	TTTGCTGCGGTGATTATGAG	Forward primer for screening present 406 bp upstream of the stop codon of *TBP1*
ONC1151	21	TGCTTATGCACAAGATTCACG	Reverse primer for screening present 196 bp downstream of the stop codon of *TBP1*
ONC12011	48	AAAAGGCCTAGAATTCCAGGTTTAATTAAAGGAGTGCAGGTGGAAACC	Forward primer with StuI site and 3bp overhangs to clone primer binding sequence (codons changed to avoid codon bias in *C. albicans*) of pFA6-*2XFKBP12-TRP1* of plasmid +FRB to pBB24
ONC12012	24	AGAATTTCACTCGGGCCCGGTACC	Reverse primer with Acc651 flanking the stop codon of 2XFKBP12 of pBB24 plasmid to clone primer binding sequence of pFA6 series of plasmid +FRB to pBB24

ONC12013	102	AAAAAGCTTAGAATTCCAGGTTTAATTAATTCAG GTGGTGGTGGTGGTTCAGGTGGTGGTTCAGGTGG TGGTGGTGCTTCAATCCTCTGGCATGAGATGTGG	Forward primer with HindIII site and 3bp overhangs to clone primer binding sequence of pFA6 -*FRB-KanMX6* of plasmid and linker used in S. cerevisiae anchor away technology (In both DNA sequences codons changed to avoid codon bias in *C. albicans*) + FRB to pBB28
ONC12015	45	AACCACCCACCAGATTTCCAGATTTCCAGAATTT CACTCGAGCTC	Reverse primer with SacI site flanking the stop codon of FRB of pBB28 plasmid to clone primer binding sequence of pFA6 -*FRB-KanMX6* of plasmid and linker used in S. cerevisiae + FRB to pBB28
ONC12018	80	GGTATCAGAGAAAAGAGAGCTAAAGAAAAGGCT GAAGCCGAAGCTGAAAAAGCTAAAAAAAGAATT CCAGGTTTAATTAA	Forward Primer for *RPL13-2XFKBP12* tagging using pBB43 as template
ONC12019	80	GAAGAAATTTATGATGCATTTGAACTGATTTATC CGGTTTTAAATGAATTTCGTAAAAATAGAATTCC AGGTTTAATTAA	Forward Primer for *TBP1-FRB* tagging using pBB50-1 as template
ONC12020	81	CGTGGTTGGGGTCATGGGAATTATACAGATACTG TATTGATTTATGAAATTGATTTCCAAATCCTCTGG CATGAGATGTGG	Forward Primer for *SPT8-FRB* tagging
ONC12021	81	TTATCGTCTGTGTGTCTGTCTGTATGTCTTTATTA TAAAATTAATGTTTTGATACCAATTGCCAGTGTG ATGGATATCTGC	Reverse Primer for deletion *SPT8* and *SPT8-FRB* tagging
ONC12022	81	AAAAACGAGTATCTGAACAAAACAAAACAAACC AAGCAATTGATTAGCAAATCACAAAGAAGCTCG GATCCACTAGTAACG	Forward Primer for *SPT8* deletion
ONC12023	81	GGAAAAAATTTATTGTGAAAATTTCCTGCATACT CTCAACCCGTAAACAACACAAAAACTAGCTCGG ATCCACTAGTAACG	Forward Primer for *TBP1* deletion
ONC12024	20	ATCTGGGGAAAATTTGGTCA	Forward primer for screening present 737 bp up stream of the stop codon of *SPT8*
ONC12025	20	ATGTGATTTGGGTGGGTGAT	Reverse primer for screening present 212 bp downstream of the stop codon of *SPT8*

ONC1249	80	CGTGGTTGGGGTCATGGGAATTATACAGATACTG TATTGATTTATGAAATTGATTTCCAAAGAATTCC AGGTTTAATTAA	Forward Primer for *SPT8-FRB* tagging using pBB50-1 as template
ONC1279	80	CAACCTGAAGGTGAATACGCTGAAAATGTGAAG AAAATTTCCAAATTAAATAAAGATCAAAGAATTC CAGGTTTAATTAA	Forward Primer for *TAF12-FRB* tagging using pBB50-1 as template

References

1. Noble SM, Johnson AD.2005. Strains and strategies for large-scale gene deletion studies of the diploid human fungal pathogen Candida albicans. Eukaryot Cell 4:298-309.
2. Cruz MC, Goldstein AL, Blankenship J, Del Poeta M, Perfect JR, McCusker JH, Bennani YL, Cardenas ME, Heitman J.2001. Rapamycin and less immunosuppressive analogs are toxic to Candida albicans and Cryptococcus neoformans via FKBP12-dependent inhibition of TOR. Antimicrob Agents Chemother 45:3162-70.
3. Haruki H, Nishikawa J, Laemmli UK.2008. The anchor-away technique: rapid, conditional establishment of yeast mutant phenotypes. Mol Cell 31:925-32.
4. Wong KH, Struhl K.2011. The Cyc8-Tup1 complex inhibits transcription primarily by masking the activation domain of the recruiting protein. Genes Dev 25:2525-39.
5. Sinha I, Kumar S, Poonia P, Sawhney S, Natarajan K.2017. Functional specialization of two paralogous TAF12 variants by their selective association with SAGA and TFIID transcriptional regulatory complexes. J Biol Chem 292:6047-6055.