

SUPPLEMENTAL MATERIAL

Supplemental Tables

Table S1. Sequences of oligonucleotides

Oligonucleotide	Sequence (5' to 3')
DNA oligonucleotides	
<i>RNR1</i> <i>XhoI</i> 5'	GCATCTCGAGAGAAATATTTCTAGCCCTGGAACGAG
<i>RNR1</i> <i>SacI</i> 3'	GTAGAGCTCTTCTCATTGAAGAACGATATAGGCGCAT
<i>GPD/TDH3</i> promoter <i>SphI</i>	GGGAGAGAGAAAAAGAAAAAGAAAAAGAAAGTTGCATGCGAAATGGC GAGTATTGATAATGATAAACTC
<i>RNR1</i> start – <i>GPD/TDH3</i> promoter	GGACAGGTTCTTTACGACCGTCTCTTTTATAAACGTACATTTTGTTTGT TATGTGTGTTTATTCGAAAC
<i>RNR1</i> start	ATGTACGTTTATAAAAGAGACGGTCGTAAGAACC
<i>RNR1</i> vector <i>XhoI</i>	CTAAAGGGAACAAAAGCTGGGTACCGGGCCCCCCTCGAGTTCTCATT GAAGAACGATATAGGCGCAT
<i>RMD9</i> knockout forward	GTGTCATTTTCTTACACTATTTCGAGAGTCCATATACTGCAAAAGGGAC AGCGTTTCGGTTGATGACGGTA
<i>RMD9</i> knockout reverse	TTATTTACAAGCATGCATAAATTGAGATGGGGAGAATGAATGCGCTAGT ACCTGATGCGGTATTTTCTCCTTTTACGC
KANB2	GCACGTCAAGACTGTCAAGG
<i>RMD9</i> knockout check	CTGTCTTTGCCTTGGTAGTTGCATCCA
probe 1105	TGCAGCACGAACTAAAGACAAC
probe 1460	ACGCGTGATGAGTGATTAGTGC
probe 1594	GGTTCCCCACGGTAACGT
RT primer	TACACGACGCTCTTCCGATCT
Illumina TruSeq Read 1 sequencing primer	ACACTCTTTCCCTACACGACGCTCTTCCGATCT
5'-truncated Illumina TruSeq Read 2 sequencing primer for <i>C.j.</i> 15S (R37265)	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGACCCTAATGTTGTGG GCTATAGACG
5'-truncated Illumina TruSeq Read 2 sequencing primer for <i>C.j.</i> <i>ATP9</i> (R4145)	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGAGCAGGGATCGGA ATTGCTATCG
5'-truncated Illumina TruSeq Read 2 sequencing primer for <i>C.j.</i> <i>COX1</i> (R25576)	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAAGGAATGCCTCGTA GAATTCCTG
5'-truncated Illumina TruSeq Read 2 sequencing primer for <i>C.l.</i> 15S (F16674)	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCGCGAACTAGAGTGTC GCGGTG
5'-truncated Illumina TruSeq Read 2 sequencing primer for <i>C.l.</i> <i>ATP9</i> (F24432)	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGATTAGGAGGAGCAG CGATAGG
5'-truncated Illumina TruSeq Read 2 sequencing primer for <i>C.l.</i> <i>COX1</i> (F2699)	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGTATGCCTCGTCGTA TACCTC
5'-truncated Illumina TruSeq Read 2 sequencing primer for <i>C.s.</i> 15S (F24836)	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGATTACTCTCTGTAAC TCGAGAG
5'-truncated Illumina TruSeq Read 2 sequencing primer for <i>C.s.</i> <i>NAD4</i> (F30205)	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGTGAATTATTAAGTC TTACTGGTGC
5'-truncated Illumina TruSeq Read 2 sequencing primer for <i>C.s.</i> <i>NAD5</i> (F37708)	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCCTCTTATCTTAGGTC TTAGTCTATCTAG
5'-truncated Illumina TruSeq Read 2 sequencing primer for <i>M.o.</i> 15S (F5789)	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAGATGGGCTATCGAC GTGTTAC
5'-truncated Illumina TruSeq Read 2 sequencing primer for <i>M.o.</i> <i>ATP9</i> (F230)	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGTGCAGGTATTAGCA CTTTAGCC
5'-truncated Illumina TruSeq Read 2 sequencing primer for <i>M.o.</i> <i>COX1</i> (F56349)	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTTAGGACTTAATGGTA TGCCTCGTAG
5'-truncated Illumina TruSeq Read 2 sequencing primer for <i>S.c.</i> 15S (F7684)	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAAGTTCATAACGAACA AAACTCCA
5'-truncated Illumina TruSeq Read 2 sequencing primer for <i>T.d.</i> 15S (F12406)	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCGCACTAATCACTCAT CACGCGTTG
5'-truncated Illumina TruSeq Read 2 sequencing primer for <i>T.d.</i> <i>COX1</i> (F25928)	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCTGATGCATTTGCAG GTTGAAC
5'-truncated Illumina TruSeq Read 2 sequencing primer for <i>T.d.</i> <i>COX2</i> (F1348)	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCTTCAGTTGTTGTAC CTGTTGATACAC

RNA oligonucleotides	
<i>Cm-15S</i>	CAUUAUCUAUUCUUUUUAUCU
<i>Cm-COX1</i>	ACUUAUCUAUUCUUUUAAU
<i>Cs-15S</i>	UAUUAUCUAUCCUUAUUCA
<i>Cs-COX1</i>	AUCUAUCUAUUCAUUAUUUA
<i>NSC</i>	UUAAUUAAUUCAAAUAUUU
<i>Sc-15S</i>	UUAAUUAAUUCUUUAUUU
<i>Sc-SCEI</i>	UUAAUUAAUUCUUUAUUU
Chimeric and modified DNA/RNA oligonucleotides	
3'-terminated DNA adapter	N ₄ AGATCGGAAGAGCGTCGTGTAAddC*
5'-adenylated, 3'-terminated DNA adapter	rAppN ₄ AGATCGGAAGAGCGTCGTGTAAddC*

* ddC - 2',3'-dideoxycytidine-5'-monophosphate residue; N₄ - four base-randomized 2'-deoxynucleoside-5'-monophosphate residues; rApp - adenosyl 5'-pyrophosphoryl.

Gene-specific sequence of each 5'-truncated Illumina TruSeq Read 2 sequencing primer is underlined.

Table S2. Putative 3'-RPEs in the Saccharomycotina subphylum of Ascomycota fungi

Species	Accession	In mRNAs		In 15S rRNA
		Consensus	Deviant sequences	
Debaryomycetaceae				
Aciculoconidium aculeatum	OR703834*	UAUm <u>UAUUC</u> wUU	ATP9 AAUCUAUUCAUU NAD1 UUUUAUAUUCAUU	AAUAUAUUCUUU
Danielozyma ontarioensis	OR703917*	Aww <u>UAUUC</u> AwGAU	NAD3 AUAUAUUCAUUGAA	AUAUAUUCUUUACU
Debaryomyces fabryi	OR703919*	Ahm <u>UAUUC</u> AwG	COB AUAUAUUCUUUG	AUAUAUUCAUUC
Debaryomyces hansenii	NC_010166	Ahm <u>UAUUC</u> AwG	COB AAAUAUUCUUUG	AUAUAUUCAUUC
Candida gorgasii	OR703877*	UAUUU <u>UAUUC</u> AU	ATP6 UAUUUAUUCUU COB UAUAUAUUCAU COX1 UAUAUAUUCAU	CAUUUAUUCUC
Candida maltosa	NC_013830	UAwh <u>UAUUC</u> yUwUA	ATP9 GAUUUAUUCUUUA	UAUCUAUUCUUUA
Candida margitis	NC_063596	AUAwh <u>UAUUC</u> hU	ATP9 UUAUAUAUUCUU	UUAUCUAUUCAU
Candida parapsilosis	NC_005253	UAww <u>UAUUC</u> wUUUU	COX2 UAUAUAUUCUUUA NAD5 UAUUUAUUCUUUU	UAUCUAUUCUUUA
Candida sojae	NC_013839	UAwy <u>UAUUC</u> AAUUAU	ATP9 UAACUAUUCAUCAU NAD5 UAUCUAUUCUUUAU	UAUCUAUUCUUUAU
Candida theae	NC_022169	UAww <u>UAUUC</u> UUUU	ATP9 UAUUUAUUCUUUU NAD4 UAUAUAUUCAAUU NAD5 UAUUUAUUCUUUU	UAUCUAUUCUUUU
[Candida] psychrophila	NC_036103	Ahm <u>UAUUC</u> AwG	none	AUAUAUUCAUUC
[Candida] railenensis	NC_063703	Ahw <u>UAUUC</u> AUhGUU	none	AAUAUAUUCAUUGUU
Hyphopichia burtonii	OR703931*	Awd <u>UAUUC</u> AUUGA	none	ACUUAUUCAUUAC
Hyphopichia gotoi	OR703878*	AUw <u>UAUUC</u> AUUUA	none	AUAUAUUCUUUCU
Kodamaea laetipori	OR703951*	AUAUAUUCAUAU	none	AUAUAUUCUUUAU
Kurtzmaniella clerdarum	OR703960*	Ahw <u>UAUUC</u> AwGUU	COX1 AAUAUAUUCAUAGGU	AAUUUAUUCUUUAUA

<i>Priceomyces medius</i>	OR704018*	Awh <u>UAUUC</u> AUAGdAU	COX3 AAAUAUUC <u>AUAGGAG</u> NAD5 AUCUAUUC <u>UAGU</u> AU	AUUUAUUC <u>UACCCAC</u>
<i>Scheffersomyces lignosus</i>	OR704029*	Ad <u>AUUUAUUC</u> AUwkU	COX3 <u>UUAUUUAUUC</u> AUUGU	<u>UGACUUUAUUC</u> CAAA
<i>Suhomyces emberorum</i>	OR703871*	UAUh <u>UAUUC</u> nUUvU	ATP9 <u>AAUUUAUUC</u> CUUCU NAD3 <u>UAUAUAUUC</u> UAGU	<u>AAUAUAUUC</u> UUA
<i>Teunomyces gatunensis</i>	OR703876*	Uw <u>UAUAUAUUC</u>	none	<u>UAAUAUAUUC</u>
<i>Teunomyces kruisii</i>	OR703885*	<u>UAUAUAUUC</u>	none	<u>AAUAUAUUC</u>
<i>Wickerhamia fluorescens</i>	OR704052*	<u>UAUAUAUUC</u> A	ATP9 <u>UAUAUAUUC</u> C COB <u>GAUAUAUUC</u> A	<u>CAUAUAUUC</u> U
<i>Yamadazyma tenuis</i>	CP117544*	UAUh <u>UAUUC</u> AUUU	ATP9 <u>UAUUUAUUC</u> AU <u>CU</u> COX3 <u>CAUCUAUUC</u> AUUU	<u>UAUUUAUUC</u> <u>UACC</u>
Dipodascaceae				
<i>Dipodascus albidus</i>	OR703925*	AwW <u>UAACUAUUC</u> C	ATP9 <u>UAAUAACUAUUC</u> C COX2 <u>AAUUAACUAUUC</u> G	<u>UCUUGACUAUCC</u>
<i>Dipodascus geniculatus</i>	OR703926*	UAwy <u>UAACUAUUC</u> CwU	ATP9 <u>UUUUUAACUAUUC</u> CU	<u>AUCUUGACUAUCC</u> CC
<i>Geotrichum candidum</i>	NC_025768	Uhd <u>UUAACUAUUC</u>	ATP9 <u>ACGUUUA</u> CUAUUC	<u>AUCUUGACUAUCC</u>
<i>Magnusiomyces capitatus</i>	NC_024095	<u>UAACUAUUC</u>	none	<u>UGACUAUUC</u>
<i>Magnusiomyces magnusii</i>	NC_020043	<u>ACUAACUAU</u> yC	NAD1 <u>UCUAACUAU</u> CC	<u>CUUGACUAU</u> CC
<i>Saprochaete ingens</i>	NC_058229	<u>UAACUAUUC</u> U	none	<u>UGACUAUCC</u>
<i>Saprochaete suaveolens</i>	NC_058213	CwCUA <u>ACUAU</u> yC	ATP9 <u>UUCUAACUAU</u> CC NAD5 <u>CACUAACUAU</u> U	<u>UCUUGACUAU</u> CC
Metschnikowiaceae				
<i>Clavispora lusitaniae</i>	NC_022161	AUw <u>UAUUC</u> AUhUU	NAD3 <u>AAUAUAUUC</u> AU <u>CU</u>	AUUUAUUC <u>AU</u> CUU
<i>Metschnikowia aberdeeniae</i>	NC_050377	AUw <u>UAUUC</u> AUwUAU	ATP9 <u>AAUAUAUUC</u> AUAU	AUUUAUUC <u>AU</u> AUAU
<i>Metschnikowia agaves</i>	NC_050343	Ahw <u>UAUUC</u> AUwwA	NAD1 <u>UAUAUAUUC</u> AUUA	AUUUAUUC <u>AU</u> UUA
<i>Metschnikowia amazonensis</i>	NC_050838	AUA <u>UAUUC</u> AUAUA	COB <u>AUAUAUUC</u> CUAUA NAD3 <u>AAUAUAUUC</u> AUAUA	AUUUAUUC <u>AU</u> CUA
<i>Metschnikowia arizonensis</i>	NC_050304	Adh <u>UAUUC</u> AUwUAU	ATP9 <u>AGUAUAUUC</u> AUA <u>A</u>	AUUUAUUC <u>AU</u> AUA
<i>Metschnikowia borealis</i>	NC_050376	AUA <u>UAUUC</u> AUAUA	ATP9 <u>AUUUAUUC</u> AUAUA NAD3 <u>AUAUAUUC</u> AUUA	AUUUAUUC <u>AU</u> CUA
<i>Metschnikowia bowlesiae</i>	NC_050844	AUA <u>UAUUC</u> AUAUAU	COX2 <u>AUAUAUUC</u> AUAUA	AUUUAUUC <u>AU</u> CUAU
<i>Metschnikowia cerradonensis</i>	NC_050344	AUA <u>UAUUC</u> AUAUA	none	AUUUAUUC <u>AU</u> CUA
<i>Metschnikowia continentalis</i>	NC_050341	AUA <u>UAUUC</u> AUAUA	none	AUUUAUUC <u>AU</u> CUA
<i>Metschnikowia dekortorum</i>	NC_050342	AUA <u>UAUUC</u> AUAUAU	COX2 <u>AUAUAUUC</u> AUAUA	AUUUAUUC <u>AU</u> CUAU
<i>Metschnikowia drakensbergensis</i>	NC_050338	AUw <u>UAUUC</u> AUwUA	none	AUUUAUUC <u>AU</u> UUA
<i>Metschnikowia drosophilae</i>	NC_050840	AUUUAUUC <u>AU</u> UUA	COB <u>AAUAUAUUC</u> AUUUA	<u>AGUAUAUUC</u> AUUUA
<i>Metschnikowia hawaiiensis</i>	NC_050340	AwA <u>UAUUC</u> AUwUA	ATP9 <u>UAUAUAUUC</u> AUAUA COB <u>AUCUAUUC</u> AUAUA	AUUUAUUC <u>AU</u> UUA
<i>Metschnikowia hibisci</i>	NC_050846	Aww <u>UAUUC</u> AUAUAU	COB <u>AUUUAUUC</u> AU <u>UUAU</u> NAD5 <u>AUUUAUUC</u> AUA <u>G</u> AU	AUUUAUUC <u>AU</u> AUAU
<i>Metschnikowia kipukae</i>	NC_050837	AwA <u>UAUUC</u> AUAUA	ATP9 <u>AAUAUAUUC</u> AUAUA	AUUUAUUC <u>AU</u> CUA
<i>Metschnikowia lochheadii</i>	NC_050374	AUA <u>UAUUC</u> AUAUA	none	AUUUAUUC <u>AU</u> CUA
<i>Metschnikowia matae</i>	NC_050382	AUA <u>UAUUC</u> AUAUA	none	AUUUAUUC <u>AU</u> CUA
<i>Metschnikowia orientalis</i>	NC_050842	UdwA <u>UAUUC</u> AUAUAU	NAD1 <u>CAUAUAUUC</u> AUAUAU	<u>UAUUUAUUC</u> AU <u>CU</u> AU
<i>Metschnikowia santaceciliae</i>	NC_050379	AUA <u>UAUUC</u> AUAUA	none	AUUUAUUC <u>AU</u> CUA

<i>Metschnikowia similis</i>	NC_050845	AUAUAUUCAUUAU	COX2 AUAUAUUCAUUA	AUUUAUUCAUUA
<i>Metschnikowia torresii</i>	NC_050839	AUUUAUUCAUUA	COB AAUAUAUUCAUUA	AGAUAUUCAUUA
[<i>Candida</i>] <i>intermedia</i>	LT635771 ^a	AUwUAUUCAUUU	ATP6 AUAUAUUCAUU	AUUUAUUCAUUU
[<i>Candida</i>] <i>ipomoeae</i>	NC_050380	AUAUAUUCAUUA	ATP9 AUUAUAUUCAUUA COX2 GUAUAUUCAUUA	AUUUAUUCAUUA
Phaffomycetaceae				
<i>Barnettozyma californica</i>	NC_022159	UhhAAUUC	none	UACAUAUUC
<i>Barnettozyma populi</i>	OR703845 ^a	UwhAAUUC <u>h</u> UU	ATP6 UUAUAUUC <u>CA</u> U NAD4 UAAUAUUCUUU	UACAUAUUC <u>CA</u>
<i>Barnettozyma pratensis</i>	OR703846 ^a	UwAAUAUUCwUU	COX3 UAAUAUUCUUC	UACAUAUUC <u>AA</u>
<i>Cyberlindnera jadinii</i>	NC_022163	UwCAUAUUCAUU	COX2 UACAUAUUC <u>AA</u> COB UUCAUAUUC <u>AC</u>	UUCAUAUUC <u>AA</u>
<i>Cyberlindnera suaveolens</i>	NC_022167	UUwUUmAUAUUCm <u>w</u> U	COB UAUAUUCUAUAUUAU	UUAUUCUAUAUUC <u>CA</u>
[<i>Candida</i>] <i>norvegica</i>	NC_022175	UUUAUAUUCwUU	ATP6 UUAUAUAUUCAUU ATP9 UAUAUAUUCUUU NAD3 CUUAUAUUCUUU	UUACAUAUUC <u>UA</u>
[<i>Candida</i>] <i>vartiovaarae</i>	NC_022164	UwUUCUAUAUUC <u>A</u>	COX1 UAUAUAUUC <u>C</u>	UUUUCUAUAUUC <u>A</u>
<i>Phaffomyces opuntiae</i>	OR704007 ^a	UwAAUAUUCwhU	none	UACAUAUUC <u>UA</u>
<i>Starmera amethionina</i>	OR704038 ^a	UAAUAUUCUhwUA	none	<u>AA</u> UAUUCUAUUA
<i>Wickerhamomyces canadensis</i>	NC_001762	UhAAUAUUCwU	none	UACAUAUUCUU
<i>Wickerhamomyces mucosus</i>	NC_022171	UUwAAUAUUCAUU	COX1 UUAUAUAUUC <u>CU</u> COX2 CUUAUAUAUUAU COX3 UUAUAUAUAUA	UUUCAUAUUC <u>AC</u>
Saccharomycetaceae				
<i>Kazachstania sinensis</i> ^b	NC_036748	UUAkUAUAUUCU	COX1 AUAUAUAUAUUCU VAR1 UAUAUAUAUAUUCU	UCUUUAUAUAUUCU
<i>Kazachstania unispora</i>	NC_036378	AUAUAUAUUCU	ATP9 AGUAUAUAUA	UUUUUAUAUUC
<i>Kluyveromyces marxianus</i>	NC_036023	UUAUAUAUUCUwU	VAR1 AUAUAUAUUCUUU	UCUAUAUAUUCUU
<i>Lachancea dasiensis</i>	HE983611	UUUkUAUAUAUAUUCU	ATP6 CAUGUAUAUAUAUUA	UAUAUCUUUAUAUAUUCU
<i>Lachancea meyersii</i>	HE983614	UdUAUAUAUAUUC <u>h</u> U	COB UUUCAUAUAUAUUCU COX3 AGUAUAUAUAUUCU	AUCUUUAUAUAUUCU
<i>Lachancea mirantina</i>	HE983613	UAUAUAUAUUCw <u>w</u> U	COB UGAUAUAUAUUCUAU VAR1 UAUAUAUAUAUUC <u>UA</u>	CUUUAUAUAUUCUUU
<i>Lachancea nothofagi</i>	HE983612	UAUAUAUAUUCUwU	ATP9 UGAUAUAUAUUCUAU	CUUUAUAUAUUCUUU
<i>Nakaseomyces bacillisporus</i>	NC_012621	UAwUAUAUAUAUUCU <u>h</u> A	ATP6 UUAUAUAUAUAUUCUU COB AAUAUAUAUAUUCUU COX1 UAUAUAUAUAUUCU <u>C</u> VAR1 UAUAUAUAUAUUC <u>UA</u>	UAUCUUUAUAUAUUCUAA
<i>Nakaseomyces braccarensis</i>	NC_036380	AUAUAUAUUCUwUAA	ATP9 AUAUAUAUUCUUUA COB AUAUAUAUAUUCUUUA VAR1 UUAUAUAUAUUCUUUA	CUUUAUAUAUUCUUAA
<i>Nakaseomyces castelii</i>	NC_012620	UwAUAUAUAUAUUCU	ATP6 UUAUAUAUAUAUUCU ATP9 UUAUAUAUAUAUUCU VAR1 AAUAUAUAUAUUCU	AUAUCUUAUAUAUUCU
<i>Nakaseomyces delphensis</i>	NC_012619	AUAUAUUCUwUAAwA	VAR1 AUAUAUUCUUUAUA	UUAUAUUCUUUAAAU
<i>Nakaseomyces nivariensis</i>	NC_036379	AwwAUAUAUUCUUUAA	ATP9 AUAUAUAUAUUCUUUA COB UAUAUAUAUUCUUUAA COX1 AAUAUAUAUUCUUA	UCUUAUAUAUUCUUUAA
<i>Naumovozyma dairenensis</i>	NC_036377	AAUAUAUUC <u>A</u>	COX2 UUAUAUAUUC <u>A</u>	UUUAUAUUCU
<i>Saccharomyces arboricola</i>	NC_031511	AAUAUAUUCU	ATP6 AAUAUAUUC <u>CA</u>	UUAUAUAUUCU
<i>Saccharomyces bayanus</i>	NC_031513	UwUAUAUAUAUUCU <u>h</u> U	ATP9 AUAUAUAUAUUCUU COX2 UAUAUAUAUUCUAC COX3 UAUAUAUAUUCUUU	AUCUUAAUAUUCUCC

<i>Saccharomyces cariocanus</i>	NC_031514	Uww AAUAAU AUUCUU	ATP9 AUUAAUAA CAUUCUU	AUCUUA AAUAAUUCUU
<i>Saccharomyces eubayanus</i>	NW_017264706	UAAUAAU AUUCUU hU	COX2 UAAUAAUAAUUCUU A C	CUUAAU AAUAAUUC CC
<i>Saccharomyces jurei</i>	LT986467*	UAAUAAU AUUCUU	ATP9 UAAUAA CAUUCUU VAR1 AAUAAU AAUUCUU	CUUAAU AAUAAUUCUU
<i>Saccharomyces kudriavzevii</i>	NC_031184	UwUAAUAAU AUUCUU	none	AUCUUA AAUAAUUCUU
<i>Saccharomyces mikatae</i>	NC_031185	Uww AAUAAU AUUCUU	ATP9 AUUAAUAA CAUUCUU	AUCUUA AAUAAUUCUU
<i>Saccharomyces paradoxus</i>	NC_018044	Uww AAUAAU AUUCUU	ATP9 AUUAAUAA CAUUCUU	AUCUUA AAUAAUUCUU
<i>Saccharomyces uvarum</i>	NC_031512	UwUAAUAAU AUUCUU hU	ATP9 AUUAAUAAU AUUCUU C COX2 UAAUAAUAAUUCUU A C COX3 UAAUAAUAAUUCUU A U	AUCUUA AAUAAUUC CC
<i>Torulaspora delbrueckii</i>	NC_027433	UAAUAAU AUUC	COX3 UGA UAAU AUUC	CUUUAU AU AUUC
<i>Torulaspora franciscae</i>	KM595069	UAAUAAU AUUCU	COX3 AAU UAAU AUUCU	CUUUAU AU AUUCU
<i>Torulaspora globosa</i>	NC_027459	AUA AU AUUCUUwU	ATP9 AUAU AUUCUU UA A VAR1 AUAU AUUC CUUAU	UUAAU AUUCUU CCC
<i>Torulaspora microellipsoides</i>	KU920678	AAUAAU AUUC hU	ATP9 AAUAA CAUUCUU VAR1 AAUAAU AUUC CC	UUUAU AU AUUCU
<i>Torulaspora pretoriensis</i>	KM595071	AAUAAU AUUCU	none	UUUAU AU AUUCU
<i>Torulaspora quercuum</i>	KY989230	UAAUAAU AUUCUU	none	CUUUAU AU AUUCU
<i>Zygosaccharomyces mellis</i>	NC_036374	UwdUAAUAAU AUUCUU	ATP9 AUAUGA UAAU AUUC VAR1 UUA AUUUA UAAUUCUU	UAU CUUUA AAU AUUC CC
<i>Zygosaccharomyces parabailii</i>	CP019506	UAwUAAUAAU AUUCU	ATP9 AUUUAU AU AUUCU COX3 UAU UGA UAAU AUUCU VAR1 UAU UUAU UAAU AUUCU	UAU CUUUA AAU AUUC CC
<i>Zygotorulaspora mrakii</i>	NW_023500906*	UAAUAAU AUUC wU	ATP9 AUUUAU AU AUUCU COB UAAUAAU AUUCU A VAR1 AAUAAU AU AUUCU	CUUUAU AU AUUCU
Saccharomycodaceae				
<i>Saccharomycodes ludwigii</i>	NC_037703	AwwAUhwUAAUAAU AUUC wU	ATP9 AAUA AUUAAU AAU AUUC AU COB AAUAU AAUAAU AUUCU A COX3 UAAU AAUAAU AUUC AU VAR1 AAAU UCAAUAAU AUUCU	AAUAU CU AUA AAU AUUC CA
Trichomonascaceae				
<i>Diddensiella caesifluorescens</i>	PPJD01000027*	UAACUU UACA	ATP9 UAACUU UACU	UAACU AUACA
<i>Diddensiella santjacobensis</i>	NC_022155	UhACU AUACA UA	COX2 UCACU AUACA AA COB UUACU AUAUA	UUACU AUACA CA
<i>Sugiyamaella lignohabitans</i>	CP014499	UhUAACUw UAC	COX1 UAUCACU AUAC	CUUU ACU AUCC
<i>Trichomonascus ciferrii</i>	MN850735	AUUUACU AUwC	NAD1 AU UA ACU AUUC	CUUA ACU AUCC
<i>Wickerhamiella infanticola</i>	OR703884	AUUUwwUUU UACU	ATP9 AUUUAAUUU UAC A NAD6 AU UAU UUUU UACU	CUUUUU UUUU UACU
Trigonopsidaceae				
<i>Tortispora caseinolytica</i>	OR704045*	UUUCAC hUA UUUUU UAGU	ATP6 UAUCACU UAUUUUU UAGU COX3 UUUCACU UAUU AUAGU	none
<i>Tortispora ganteri</i>	OR704046*	UUUUACAC yUA UUUUU UAGU	ATP9 UUAUC ACU UAUUUUU UAGU COB AUUUC ACU UAUU AUAGU	none

The nucleotides comprising 3'-RPE consensus in each species are shown in *bold*. Nucleotide positions deviant from the consensus are in *red*. Variable nucleotides within 3'-RPE boundaries are in *lower case* and annotated according to the standard nucleotide nomenclature ('d' - A, G, or U; 'h' - A, C, or U; 'k' - G or U; 'm' - A or C; 'r' - A or G; 'v' - A, G, or C; 'w' - A or U; 'y' - C or

U). The common motif UAUUC found in most 3'-RPEs is *underlined*. Species analyzed in this work are highlighted in *green*.

^a Annotated with MFannot (Kehrein et al. 2015)

^b Putative 3'-RPEs in *Kazachstania sinensis* have been previously proposed (Szaboova et al. 2018), however some of them differ from the ones reported in this work.

Table S3. Occurrence of putative 3'-RPEs inside continuous ORFs.

Species	ORF	Sequence of 3' end (5' to 3')
<i>Debaryomycetaceae</i>		
<i>Aciculoconidium aculeatum</i>	NAD1	UGGUUUUAUAUUCAUUUUAUCUUGAACCUCUUUGUCAAGAUGUACCAACCGGAAGGGAAAAUUUUAUUUCCACCCU AUUUUUGACCUAGAGGGUCAUAUUCAUUCACGAGUUC CCUCCCCC CAUACUCUCGUAUGAGGUUCUUUAUUGAGGGAUUAU ACAACUAUUCGUGGCUGAAGGGUGUUAUUACAUAAGAU UUGGUUAUACUUUUCAAUUAU <u>UAG</u>
	NAD4	AUUAUAUAUAUUCAUUUAA
	NAD5	UUUAUAUAUAUUCAUUCAUCUCUACCACCCGUGGUCG GGAUAA
<i>Debaryomyces hansenii</i> *	NAD1	GGAUACCUAUUUCAUAGAAUAA
	NAD4	UUUAUAUAUAUUCAUAGUAUACCGUACUUUUUAUUAAAA UAUCCAUCCCAUGAUUAUAA
	NAD5	CUUUUAUCUAUUCAUUGUUUUUUUUUUUUUAUAUAAAAU AUUUUUUAUAUAUUCGCGUACGCGGGGAAGAAAAUU AUUAUAUAUUUUUAUAUACAAAAUGUAUAAUUAACA UUUCAUACGCCAGAGGCGGGCAUUAUCUAA
<i>Danielozyma ontarioensis</i>	NAD4	UUUAUAUAUUCAUUGAUUUUAUUAA
	NAD5	UUUAUUUAUUCAUUGAUAAAAUAUUUUUAUAAGGU AA
<i>Candida maltosa</i> *	NAD4	AUUAUAUAUAUUCUUAUAG
<i>Candida margitis</i>	NAD4	UAUUUAUAUAUAUUCAUUAA
<i>Candida parapsilosis</i>	NAD4	AUUAUAUAUAUUCAUUUUAAUAUAUAUAUAUAUAUA UAA
	NAD5	AUUAUAUUUAUUCUUCUUUUUAUAGGUAG
<i>Candida sojae</i>	NAD4	ACUUUAUUUAUUCAUUAUAUGAAUAAGUUAUAUAUA ACUAUAUAA
	NAD5	UCUUUAUCUAUUCUUAUUCUUAAUAUCUCUUUAUGU UAUAUCUAUUAUCCAUGCUAUUAG
<i>Candida theae</i>	NAD4	AUUAUAUAUAUUCAUUUAA
	NAD5	AUUAUAUUUAUUCUUCUUUUUAUAG
	NAD1	GGAUACCUAUUUCAUUGAAUAUUAA

<i>[Candida] psychrophila*</i>	NAD4	UUAUUAUAUUCAUAGUUUUUUA
	NAD5	CUUUUUCUAUUCAUUGUUUUGUACCUUUAUUAUUUAUU UAUAUAUUUAUAUAUAUAUAUGAAUAUUUAUAUAUA UAUAUUCUCCACAGGGAAAGAUUAUAUGCCCGCUUCG CGGGCAUAUAUUUAUAUUACUAAUUUUUAUACCCUGU CCAAUGGGCAGGGCAUAGGUUAUAUAUA
<i>[Candida] railenensis</i>	NAD1	GCUAAUUUAUUCAUUGUUAUCUUUUAUUUAUUUAUU UUUAUAUAUUUAUUUAUACUUUUUUUAUAAAUUAUA
	NAD3	AACUUAUAUUCAUUGUUA
	NAD4	UUAUUAUAUUCAUUGUUA
	NAD5	AUUUAUAUAUUCAUUGUUAUACUUAUUUAUUUAUA GAUA
<i>Hyphopichia burtonii*</i>	NAD1	GCUUAUAUAUUCAUUGAUUUUAUCCUCAGAAUAUCC UUUCUAGCAGUCAACAUUACUA
	NAD4	UAUUUAUAUAUUCAUUGAUUUUAUAGCAACUAACUU UUGUUGUA
<i>Hyphopichia gotoi*</i>	NAD1	GCUUAUAUAUUCAUUUUAUUUAUAUUUAUUUCUUCUU UUAAUAUAAUCUUCUUUAUAAAUUAUAUUUAUAGGUA A
	NAD4	UUAUUAUAUUCAUUUUAUA
	NAD5	AUUUAUUUAUUCAUUUUAUAUAUA
<i>Kodamaea laetipori</i>	NAD1	GGUAUAUAUUCAUUUUUCCCCCUCUCCCAUAUUA UAUUCUCCUGGCCGAUGGACAGCGAUUAUUUAUUUC GAAGAUCGCCAAUAUAUCUUUUAGUAUAUA
	NAD4	UUAUUAUAUUCAUUUUUGCCCCUAUUCUUAUUUAUA UUCUUA
	NAD5	AUAUAUAUAUUCAUUAUAUAUAUUCUCCUACAGAGAAG CCAAUAUA
<i>Kurtzmaniella cleridarum</i>	NAD1	CUCAUAUAUUCAUUGUUCUUCUUCUUUUUAUUCUUA UUCUUAUUUACCUUACUUUAUCUUACUUUUUAUUUAC CCCCUUUAUAUUUUUAUUUAUUUUUAUACCUAUUUU ACCCUAUAUAUCUUACUUAUAUUUUACCCUUUAUAUU UAUA
	NAD5	AUUUAUAUAUUCAUUGUUAUAUAUAUAUAUAUA
<i>Priceomyces medius</i>	NAD1	UCUUUAUAUAUUCAUAGUAUAUAUAUAUAUAUAUA AUUAUAAAUAUAUAUAUAUAUAUAUAUAUAUAUA A
	NAD4	UUAUUAUAUAUUCAUAGUAUAUAUAUAUAUAUAUA UAUAUAUAUAUAUAUAUAUAUAUAUAUAUAUAUAUA
	NAD5	UUAUUUCUAUUCUAUAUAUAUAUAUAUAUAUAUAUA
<i>Teunomyces gatunensis*</i>	NAD4	UUAUUAUAUAUAUUCGUAUUUAUAUAUAUAUAUAUA UAUAUAUAUAUAUAUAUAUAUAUAUAUAUAUAUAUA
<i>Teunomyces kruisii*</i>	NAD4	AUUAUAUAUAUUCGUAGAAAGGUAA
<i>Suhyomyces emberorum*</i>	NAD1	UGCUAUAUAUUCGUUAUAUAUAUAUAUAUAUAUAUA
	NAD4	AUUAUAUAUAUUCGUUAUAUAUAUAUAUAUAUAUA UAUAUAUAUAUAUAUAUAUAUAUAUAUAUAUAUAUA
	NAD5	AUUAUAUAUAUUCAUUGUAUAUAUAUAUAUAUAUA

[illegible]

Conserved and deviant nucleotide positions in putative 3'-RPEs are shown in *blue* and *red*, respectively. Stop codons are highlighted in *yellow*. *Asterisks* indicate species where ORFs overlapping with the downstream tRNA were also found (see **Supplemental Table S4**). tRNA sequences are *underlined*.

Table S4. Occurrence of overlapping between ORFs and tRNAs.

Species	ORF/tRNA	Sequence of 3' end (5' to 3')
Debaryomycetaceae		
<i>Debaryomyces hanseii</i>	COX2/tRNA ^{Asn}	CAAGAAAAUGUUUAUGUAGCUUAAAGGCAAAGC
<i>Candida maltosa</i>	COX2/tRNA ^{Asn}	UAAUGCUAAGUCU <u>UAG</u> UAGCUCAAU
<i>[Candida] psychrophila</i>	COX2/tRNA ^{Asn}	CAAGAAAAUGUUUAUGUAGCUUAAAGGCAAAGC

<i>Hyphopichia burtonii</i>	<i>COX2/tRNA^{Asn}</i>	UUGUUUU <u>AUGUUUAUGUAGCUUAAUGGUAAAAGC</u> AAUGUACUGUUAAUACA <u>UUGAUCUUGGUUCAAU</u> UCCAGGCU <u>UAA</u> ACGUUUGUU
<i>Hyphopichia gotoi</i>	<i>COX2/tRNA^{Asn}</i>	AAAUAAUAGUUUAUGUAGCU <u>UAA</u> UGGUAAAAGC
<i>Priceomyces medius</i>	<i>COX2/tRNA^{Asn}</i>	CAAGAUUGUGUUUAUGUAGCU <u>UAA</u> UGGUAAAAGC
<i>Scheffersomyces lignosus</i>	<i>COX2/tRNA^{Asn}</i>	GUUGACUUAGCUUCUGUAGCU <u>UAA</u> UGGUAAAAGC
<i>Suhomyces emberorum</i>	<i>COX2/tRNA^{Asn}</i>	AUCGAUAUAGCUUUUGUAGCU <u>UAA</u> UUGGUAAAG
<i>Teunomyces gatunensis</i>	<i>COX2/tRNA^{Asn}</i>	AUCGAAAUUGCUUUUGUAGCU <u>UAA</u> AGGUAAAAGC
<i>Teunomyces kruisii</i>	<i>COX2/tRNA^{Asn}</i>	AUCGAAAUUGCUUUUGUAGCU <u>UAA</u> AGGUAAAAGC
<i>Yamadazyma tenuis</i>	<i>COX2/tRNA^{Asn}</i>	AAGAAUAUUGUUUAUGUAGCUUAAAGGUUAAAG CAAUGUACUGU <u>UAA</u> UACA <u>UUGAU</u>
<i>Metschnikowiaceae</i>		
<i>Metschnikowia agaves</i>	<i>NAD3/tRNA^{Arg}</i>	UCU <u>UCCUCUUAUCUUAUAGUU</u> <u>UAA</u> UGGTTAGAA

Stop codons of indicated ORFs are highlighted in *yellow*. Previously annotated tRNA sequences are *underlined*.

Supplemental Figures

Saccharomyces cerevisiae

AI5β AUUAAAUAAUUCUUUUUU (39)
ATP6 UUUUAAUAAUUCUUUUUAU (86)
ATP9 AAUUAAUACAUCUUAUAA (83)
COB CUUUAAUAAUUCUUAAAA (97)
COX1 UAAUAAUAAUUCUUAUAA (68)
COX2 UUAUAAUAAUUCUUAAAU (62)
COX3 UUAUAAUAAUUCUUACAA (100)
ORF1 UUUUAAUAAUUCUUUUUAU (157)
SCE1 UUAAAUAAUUCUUAUUU
VAR1 AUAAAAUAAUUCUUAAAU (579)

Naumovozya castelii

ATP6 UAUUAAUAAUUCAUCUU (80)
ATP9 UAUUAAUAAUUCAUAU (26)
COB UAAUAAUAAUUCAUACU (26)
COX1 UAUAAUAAUUCAUAU (25)
COX2 UAUAAUAAUUCCAUAU (28)
COX3 UAUUAAUAAUUCAUAU (38)
VAR1 AAAUAAUAAUUCUAAC (19)
ψORF1 UAAUAAUAAUUCAUACU (17)
ψCOB AUAAUAAUUCAUACA (25)

Nakaseomyces glabratus
 ([*Candida*] *glabrata*)

ATP6 AUAAUAAUUCUUAAU (29)
ATP9 AUAAUAAUUCUUAAUA (33)
COB UAUAAUAAUUCUUAACU (22)
COX1 UAAUAAUAAUUCUUAAU (20)
COX2 UAUAAUAAUUCUUAUUU (107)
COX3 UAUAAUAAUUCUUUAUU (14)
VAR1 AUAAAAUAAUUCUUUAUA (320)

Kazachstania servazzii

ATP6 GAAAUAAUUAUUCUCCC (179)
ATP9 AAGAUAAUUAUUCAAUC (35)
COB AUUCUAAUUAUUCUUA
COX1 AAUCUAAUUAUUCUUAC
COX2 AUAAUUAUUCUAUC (20)
COX3 CAUAUAAUUAUUCAUUU (28)
ORF1 UUAUUAAUUAUUCUAUU (31)
ORF1 AGAAUAAUUAUUCAAUC (142)
VAR1 UAAAUAAUUAUUCAUAC (120)

Lachancea thermotolerans
 (*Kluyveromyces thermotolerans*)

ATP6 CAUGUAAUAAUUCUAUUU (47)
ATP9 ACUAGAUAAUUCUUAUU (36)
COB AUAAUAAUUCUUUU (42)
COX2 UUAGUAAUAAUUCUCCCU (75)
COX3 UAAGUAAUAAUUCUAUUC (26)
VAR1 UAUGUAAUAAUUCUAUUU (67)
COX1 AUUUAAUUAUUCUUAUU (23)*

Figure S1. Supplemental Data for Figure 1A. Gene-specific RNA sequence fragments used to generate the 3'-RPE sequence logos shown in **Figure 1A**. For each species, positions matching the consensus are shown in *blue* and those deviating from the consensus are in *red*. The distances between the 3'-RPEs and upstream ORF stop codons are indicated in parentheses, while stop codons within the 3'-RPEs are *underlined*. Although the presence of “dodecamer-like”

sequences have been suggested previously in these organisms (Clark-Walker et al. 1985; Talla et al. 2005; Kehrein et al. 2015), their boundaries may differ from those presented here for 3'-RPEs. A putative deviant 3'-RPE in the *L. thermotolerans* COX1 mRNA (asterisk) has not been reported previously (Talla et al. 2005). Accession numbers of the genomic sequences used are as follows: NC_001224 (*S. cerevisiae*), NC_004691 (*N. glabratus*), NC_006626 (*L. thermotolerans*), NC_003920 (*N. castellii*), NC_004918 (*K. servazzii*).

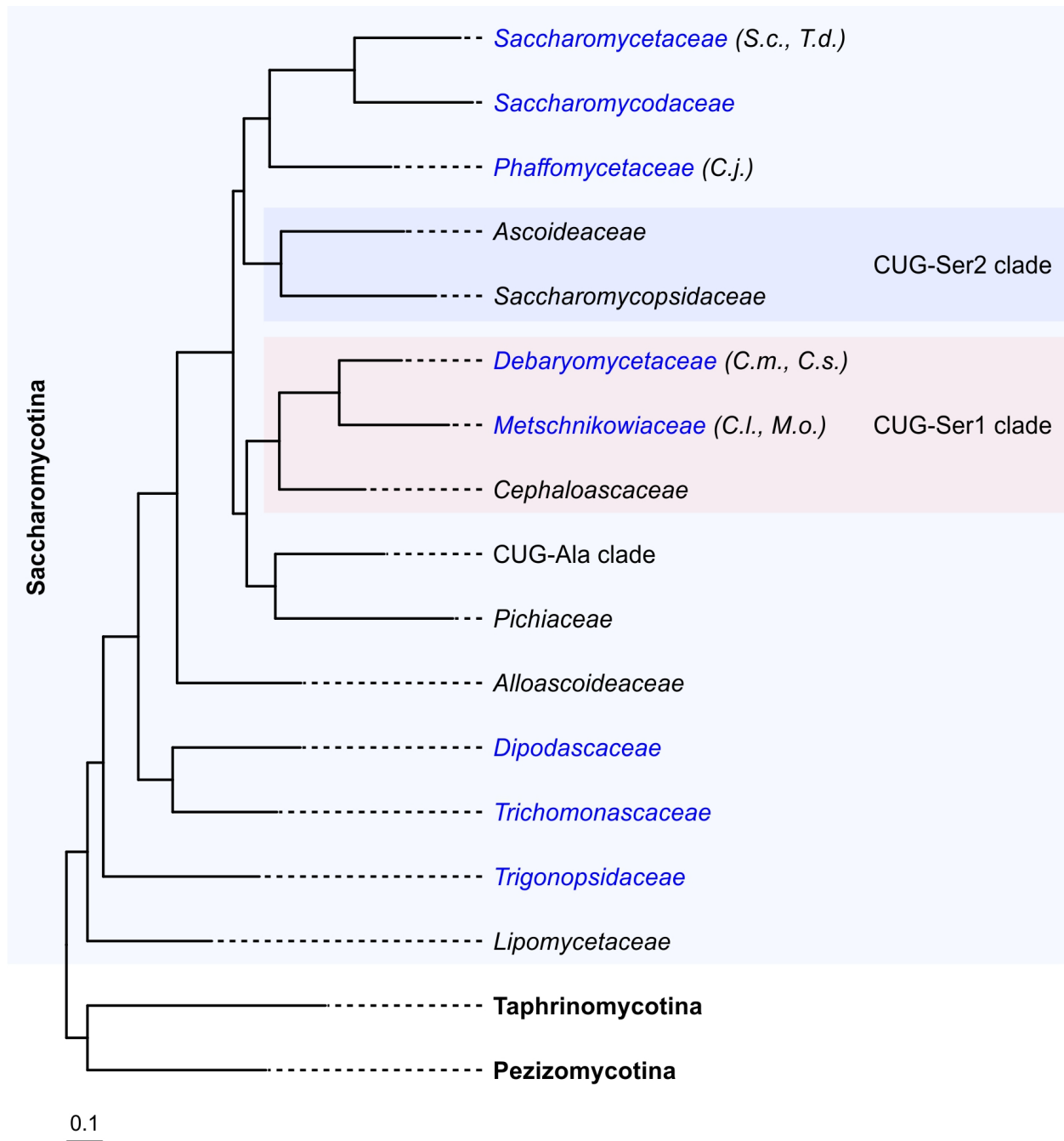


Figure S2. The phylogeny of lineages in the subphylum *Saccharomycotina*. The phylogenetic tree was inferred from the 1687 single-copy orthologs identified by BUSCO (v. 5.1.2; genome mode with the lineage *ascomycota_odb10*; (Manni et al. 2021)) in at least 50% of the reference genome assemblies of a representative species in each lineage, i.e. *Alloascoidea*

africana (GCA_030581615.1), *Ascoidea rubescens* (GCA_001661345.1), *Candida auris* (GCA_003013715.2; *Metschnikowiaceae*), *Cephaloascus fragrans* (GCA_003707825.3), *Debaryomyces hansenii* (GCA_000006445.2), *Dipodascus albidus* (GCA_003707475.1), *Lipomyces starkeyi* (GCA_018804115.1), *Nakazawaea holstii* (GCA_003706265.2; CUG-Ala clade), *Phaffomyces opuntiae* (GCA_003707165.2), *Pichia membranifaciens* (GCA_001661235.1), *Saccharomyces cerevisiae* (GCA_000146045.2), *Saccharomycodes ludwigii* (GCA_020623625.1), *Saccharomycopsis javanensis* (GCA_031125485.1), *Trichomonascus ciferrii* (GCA_030573635.1), and *Trigonopsis californica* (GCA_030574475.1). *Aspergillus nidulans* (GCA_000011425.1) and *Schizosaccharomyces pombe* (GCA_000002945.2) representing subphyla *Pezizomycotina* and *Taphrinomycotina*, respectively, were used as an outgroup. The supermatrix was calculated using public domain BUSCO_phylogenomics created by Jamie McGowan at Earlham Institute (United Kingdom) (https://github.com/jamiemcg/BUSCO_phylogenomics), and the tree was built using FastTree (2.1.11 (Price et al. 2010)). Families where putative 3'-RPEs were identified are highlighted in blue. The positions of the species studied in this work are indicated as follows: *C.m.* - *Candida maltosa*, *C.l.* - *Clavispora lusitaniae*, *C.s.* - *Candida sojae*, *M.o.* – *Metschnikowia orientalis*, *S.c.* - *Saccharomyces cerevisiae*, *T.d.* - *Torulaspora delbrueckii*.

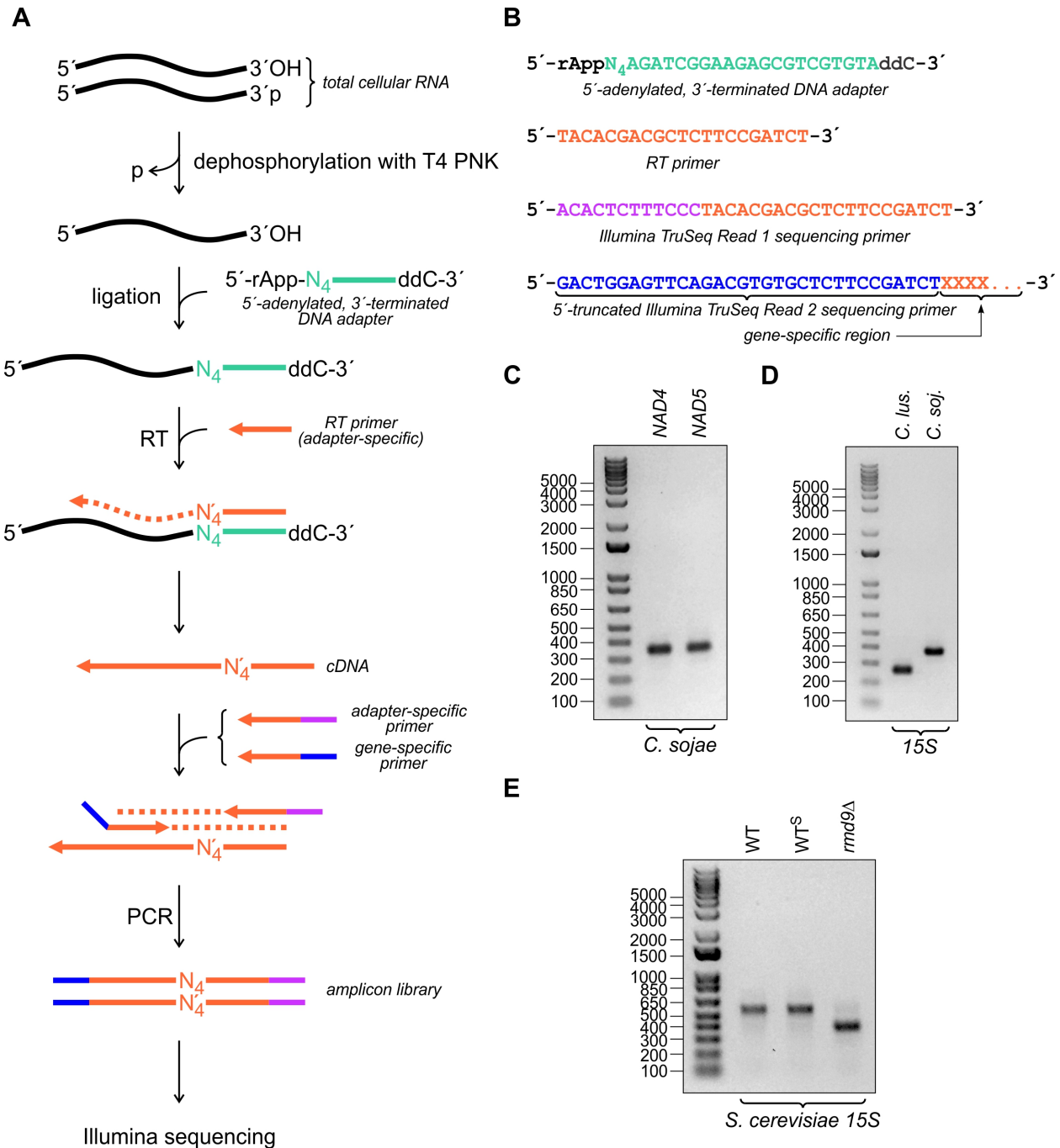


Figure S3. Explanation of the 3' RACE experiments. (A) A workflow chart for the generation of DNA amplicons from the 3'-end regions of target RNAs. Total RNA (black lines) was isolated from cells grown in media containing 2% (w/v) galactose as the carbon source. After the treatment with T4 PNK (optional for *S. cerevisiae*), 3' ends were ligated to 5'-adenylated and ddC-terminated

3' DNA adapter (*green* lines). The adapter included a four-nucleotide degenerate sequence (N₄) to reduce sequence bias in RNA recovery upon ligation (Giraldez et al. 2018). cDNA was synthesized by reverse transcription (RT) from a primer complementary to the adapter (both primer and DNA product are shown in *orange*). Amplicons were generated by PCR using adapter- and gene-specific primers, with cDNA as the template. The primers encompassed partial sequences of *Illumina TruSeq Read 1* and *Illumina TruSeq Read 2* sequencing primers (shown in *magenta* and *blue*, respectively). Primer sequences complementary or identical to cDNA regions are shown in *orange*. The amplicons were submitted to Illumina next-generation sequencing. **(B)** Primer sequences used in the experiment. Different primer regions are colour-coded as in panel A. **(C)** A 1% (w/v) agarose gel showing *NAD4*- and *NAD5*-specific amplicons from *C. sojae* (*C. soji*) total RNA submitted for next-generation sequencing (see **Figure 2C**). Numbers (in bp) indicate the sizes of dsDNA markers. **(D)** 15S rRNA-specific amplicons from *C. lusitaniae* (*C. lus.*) and *C. sojae* (*C. soji.*) total RNA were resolved and presented as in panel C (see **Figure 4**). **(E)** *S. cerevisiae* 15S rRNA-specific amplicons generated from WT, WT^S and *rmd9Δ* strains (see **Figure 7A**).

A

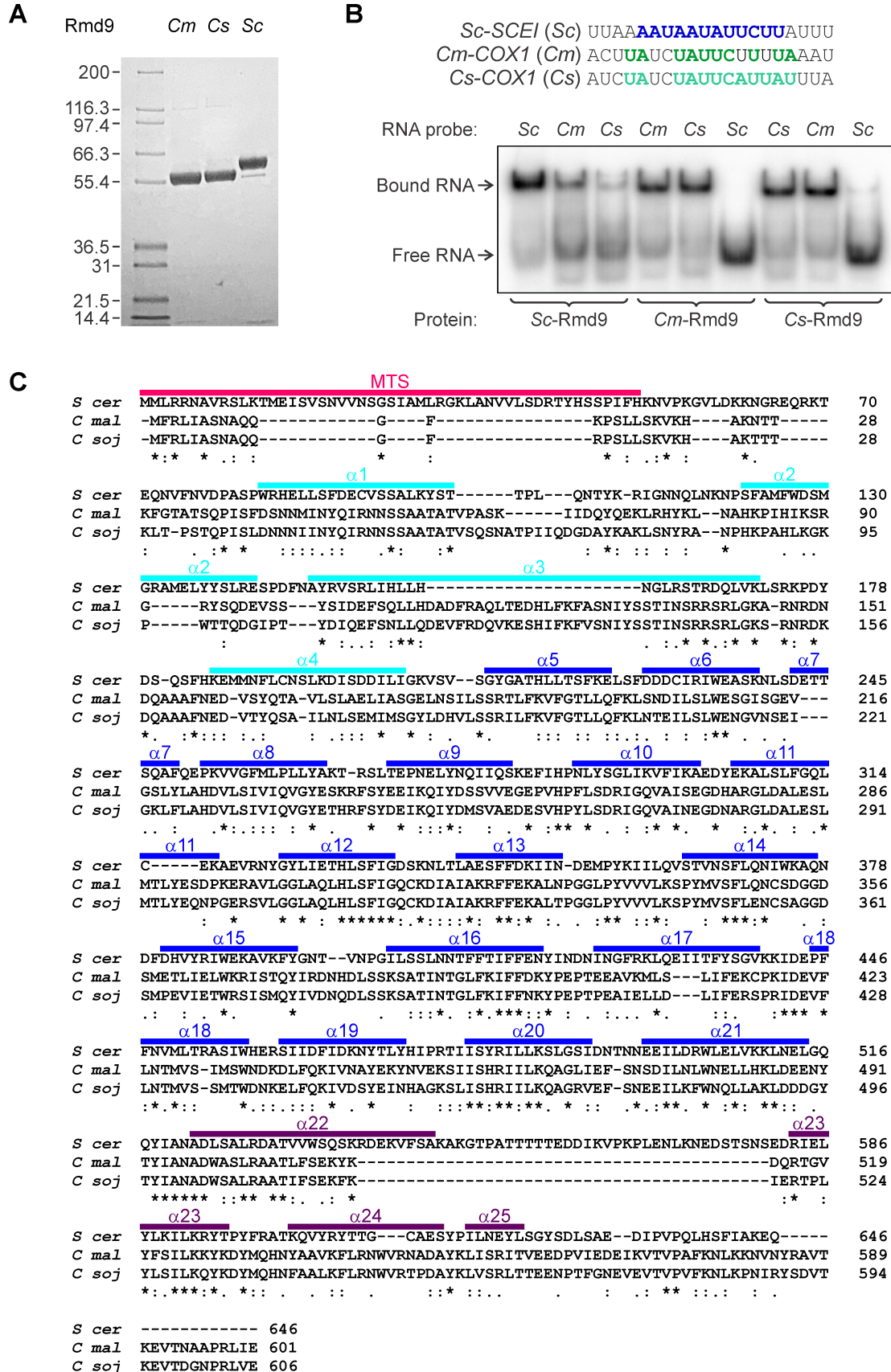


Figure S5. Rmd9 cross-reactivity with 3'-RPEs from different yeast species. (A)

Polypeptides from 2 µg aliquots of recombinant *S. cerevisiae* (Sc), *C. maltosa* (Cm) and *C. sojae* (Cs) Rmd9 protein isolates were resolved on denaturing 4-12% polyacrylamide gel and stained by Coomassie. Numbers indicate molecular weight of protein markers (kDa). **(B)** Rmd9 proteins from *S. cerevisiae* (Sc-Rmd9), *C. maltosa* (Cm-Rmd9) and *C. sojae* (Cs-Rmd9) were tested for the ability to form complexes with each other's 3'-RPE consensus sequences using EMSA. Sequences of the radioactive RNA probes with 3'-RPEs from the corresponding ORFs (Sc-SCEI or Sc, Cm-COX1 or Cm, Cs-COX1 or Cs) are shown. The unique 3'-RPE consensus nucleotides for each species are colored. In all lanes, the RNA probes and proteins were present at concentrations of 100 nM and 200 nM, respectively. **(C)** Amino acid sequence alignment showing homology of *S. cerevisiae* Rmd9 with its orthologs from *C. maltosa* and *C. sojae*. The last amino acid position of each aligned fragment is indicated; identical positions are marked by *asterisks*, strongly similar residues – by *colons*, and weakly similar residues – by *periods*. The sequence of mitochondrial targeting signal (MTS) in *S. cerevisiae* was mapped previously (Hillen et al. 2021). Alpha helices above the alignment corresponds to the structure of *S. cerevisiae* Rmd9 (PDB 7A9W). Secondary structures forming *lid*, *PPR* and a distinct *C-terminal domain* are highlighted in *turquoise*, *blue*, and *magenta*, respectively (Hillen et al. 2021). Yeast species are abbreviated as follows with the corresponding accession numbers listed in parentheses: *S. cerevisiae* (S cer, P53140), *C. maltosa* (C mal, EMG45446), *C. sojae* (C soj). *C. sojae* Rmd9 amino acid sequence was derived from contig LMTL01000173, residues 41919-43739 using translation Table 12.

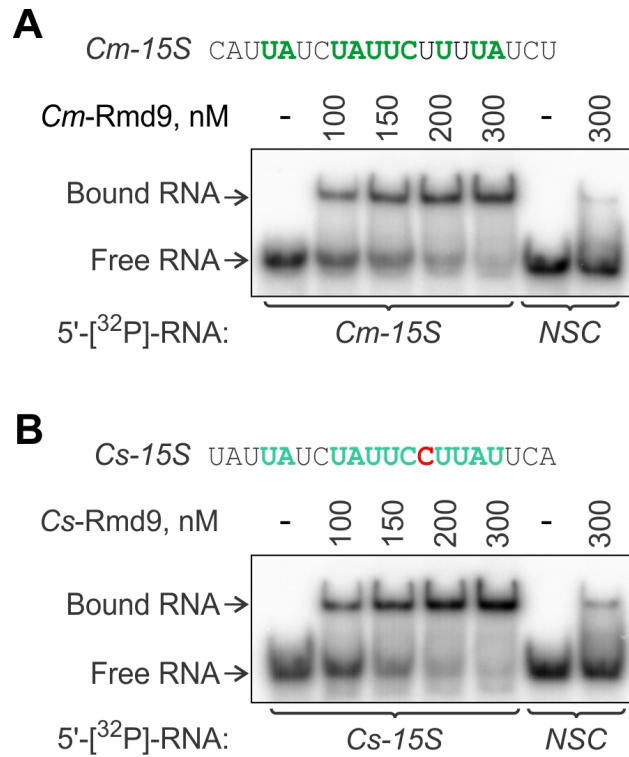


Figure S6. *S. cerevisiae* Rmd9 orthologs from the *Debaryomycetaceae* family recognize 3'-RPEs predicted to be associated with 15S rRNA processing *in vitro*. An EMSA experiment was conducted as described for **Figure 3B**.

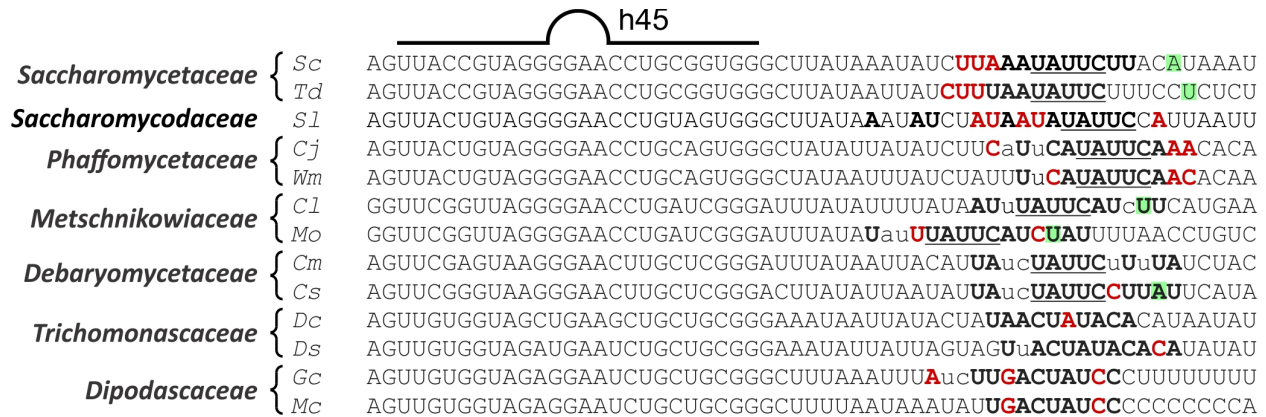


Figure S7. Structural alignment of 15S rRNA 3'-terminal regions from the species representing different families of the Saccharomycotina subphylum. The RNA sequences encompass a small subunit rRNA conserved element *helix 45* (h45) together with downstream regions. The horizontal lines above the sequences denote complementary regions of the stem, while the semicircle indicates the loop position. The nucleotides comprising the 3'-RPE consensus for each species are shown in *bold*. The nucleotide positions deviant from the consensus are shown in *red*. Variable nucleotides within 3'-RPE boundaries are in lower case. Experimentally mapped 3'-terminal nucleotides are highlighted in *green* ((Thalenfeld et al. 1983) and this work). The predominant 3'-RPE sequence motif UAUUC is *underlined*. Species abbreviations and GenBank accession numbers are as follows: *S. cerevisiae* (Sc, NC_001224); *T. delbrueckii* (Td, NC_027433); *Saccharomyces ludwigii* (Sl, NC_037703); *C. jadinii* (Cj, NC_022163); *Wickerhamomyces mucosus* (Wm, NC_022171); *C. lusitaniae* (Cl, NC_022161); *M. orientalis* (Mo, NC_050842); *C. maltosa* (Cm, NC_013830); *C. sojae* (Cs, NC_013839); *Diddensiella caesifluorescens* (Dc, PPJD01000027); *Diddensiella santjacobensis* (Ds, NC_022155); *Geotrichum candidum* (Gc, NC_025768); *Magnusiomyces capitatus* (Mc, NC_024095). The position of 15S rRNA in *Gc* was determined by MFannot (Lang et al. 2023).