

ITS1

		20		40	
K. lactis	AAGATTATGA	AT	-----	-----	12
S.cerevisiae	AAGAAATTTA	ATAAATTTTGA	AAATCGATTT	TTTTCTTTTG	40
		60		80	
K. lactis	-----GACTAG	A-----TTA	CTGGGGGA	-----	30
S.cerevisiae	GCAAGACCAA	AGAGCTTTTA	CTGGGCAAGA	AGACAAGAGA	80
		100		120	
K. lactis	---ATCGCTG	AATATGGCCT	GCGCTTAATT	GCGCGGCT	65
S.cerevisiae	TGGAGACTCC	AGCCGGGCCT	GCGCTTAAGT	GCGCGGTCTT	120
		140		160	
K. lactis	---AATTCT	TGATTTTCT	-----GCTATC	-----	86
S.cerevisiae	GCTAGGCTTG	TAAGTTTCTT	TCTTGCTATT	CCAAACGGTG	160
		180		200	
K. lactis	--AGTTTTCT	TTCTCTC	-----	-----	101
S.cerevisiae	AGAGATTTCT	GTGCTTTTGT	TATAGGACAA	TTAAAACCGT	200
		220		240	
K. lactis	---ATCCTA	AACACAAATGG	AGTTTTT-TC	TCTATGAAC	136
S.cerevisiae	TTCAATACAA	CACACTGTGG	AGTTTTCATA	TCTTTGCAAC	240
	↑A2	260		280	
K. lactis	ACTTCCCTGCG	AGAGCTCGTCT	TC-----T	CCAGTGCACA	169
S.cerevisiae	TTTTTCTTTG	GGCATTCTGAG	CAATCGGGGC	CCAGAGGTAG	280
		300		320	
K. lactis	TAAACACAAA	CAACATTTTGT	CATT-----	-----	193
S.cerevisiae	CAAACACAAA	CAATTTTATC	TAATCAATTA	ATTTTTGTCA	320
		340		360	
K. lactis	---ATGAAAA	ACTATTTATC	AAGAAATTT-	---AATATTCA	227
S.cerevisiae	AAAACACAGAA	TTTTCTGTAAC	TGGAAATTTT	AAAAATATT-A	359

ITS2

		20		40	
K. lactis	CTCTCTCAAA	CCTTTGGGTT	TGGTAGTGAG	TGATACTCTG	40
S.cerevisiae	TCCTTCTCAA	ACATTCTGTT	TGGTAGTGAG	TGATACTC	38
		60		80	
K. lactis	TTTTTGGGTT	AACCTTGAAAG	TGGCTAGCCG	TTGCCCTTCTG	80
S.cerevisiae	-TTTGGAGTT	AACCTTGAAAT	TG-CTGGCCT	TTTCATTGGA	76
		100		120	
K. lactis	CGT-----	---GAGCAGG	GCTGCGTGTC	AAGTCTATGG	110
S.cerevisiae	TGTTTTTTTT	TCCAAAGAGA	GGTTTCTCTG	CGTCTTGAG	116
		140		160	
K. lactis	ACTCGACTCT	TGCACATCTA	CGTCTTAGGT	TTGCGCCAAT	150
S.cerevisiae	GTATAATGCA	AGTACGGT--	CGTTTTAGGT	TT-TACCAAC	153
		180 C2		200	
K. lactis	TCTGTGCTAAG	CTAGGGTCAA	TGAGCTTATA	GGTGTATATA	190
S.cerevisiae	TGCGGCTAAT	CT-----TTTT	TTATACTGAG	CGTATTGGA	189
		220		240	
K. lactis	AGACTCGCTG	GTCTTTGTCT	CCTTGAGGCA	TACGGCTTAA	230
S.cerevisiae	CG-----	---TTATCG	ATAAGAAAGAG	AGCGTCTAGG	217
K. lactis	TCAAAACTCT	CAAAGT			246
S.cerevisiae	GAACCAATGT	TCTTAA			233

Supplementary Figure 1. Design of hybridization probes for *Kluyveromyces lactis* ITS1 and ITS2. The sequences of ITS1 and ITS2 in *K. lactis* was aligned with the corresponding spacers in *S. cerevisiae* using CLC Sequence Viewer 8. The arrow below the alignments indicate the 3' end points after cleavage at A2 and C2 in the *S. cerevisiae* ITS1 and ITS2, respectively (van Nues et al. 1994; van Nues 1995). The red lines above the *K. lactis* sequences indicate the sequences that are complementary to the *K. lactis*-specific probes. Note that the *K. lactis* ITS1 probe spans two insertions in *S. cerevisiae* ITS1 that prevent hybridization of the *S. cerevisiae* ITS1 to the probe.

References for supplement

- van Nues RW, Rientjes JM, van der Sande CA, Zerp SF, Sluiter C, Venema J, Planta RJ, Raue HA. 1994. Separate structural elements within internal transcribed spacer 1 of *Saccharomyces cerevisiae* precursor ribosomal RNA direct the formation of 17S and 26S rRNA. *Nucleic Acids Res* **22**: 912-919.
- van Nues RW, Rientjes, J.M.J., Morrè, S.A., Mollee, E., Planta, R.J., Venema, J., Rauë,H.A. 1995. Evolutionarily conserved structural elements are critical for processing of internal transcribed spacer 2 from *Saccharomyces cerevisiae* precursor ribosomal RNA. *J Mol Biol* **250**: 24-36.