Supplementary Information

Name:	MW: (kDa)	Type:	Cross-linked?	Mapped?	Cross-links to:	Co-IP:	References:
Dim1	36	SAM methyl transferase	Yes	Yes	18S	5'ETS, 20S	(Lafontaine et al, 1995)
Dim2	30.3	KH	Yes	No		20S	(Vanrobays et al, 2004)
Rio1	56	Serine kinase	No				(Vanrobays et al, 2004, Schafer, 2003 #334)
Rio2	49	Serine kinase	Yes	Yes	18S	20S	(Schafer et al, 2003)
Nob1	51.7	PIN domain, Zinc ribbon	Yes	Yes	18S	20S	(Fatica et al, 2003; Schafer et al, 2003)
Ltv1	53.4		Weakly	Yes	18S	20S	(Loar et al, 2004; Schafer et al, 2003)
Enp1	55.1		Yes	Yes	18S	5'ETS, 20S	(Chen et al, 2003; Schafer et al, 2003)
Tsr1	91	GTP binding	Yes	Yes	18S	5'ETS, 20S	(Gelperin et al, 2001; Schafer et al, 2003)

Supplementary Table 1. Proteins tested for cross-linking.

All proteins that were cross-linked and mapped are described in the main text.

Name:	Sequence:
Rio1-HTPF	5'-TCAAGAAGCACATCAAGAAAAAATTGGTGAAAAAAACGAAATCAAAGAAA-gagcaccatcaccatcacc-3'
Rio1-TAPR	5'-GTTCTTCGACTCCAAACAACGATTCCCAAATGTATTTTCACAGGGCCGCA-tacgactcactataggg-3'
Rio2-HTPF	5'-GTGGTGTTGAAAATCTAAAAATGGATAAACTAGGAAACTATATACTAGAG-gagcaccatcaccatcacc-3'
Rio2-TAPR	5'-GGATAACAACTTGATTATTTGCGGCCATTTATGCAGTCGTCTAAACTAAA-acgactcactataggg-3'
Dim1-HTPF	5'-ATTTCCTAAGGCTATTATATGCTTTTCACCAGGTTGGTATCCATTTTTCA-gagcaccatcaccatcacc-3'
Dim1-TAPR	5'-CTTATCTTAGGTAAATAGTATACAAGCACTTACATAATTGATAAGAGAGC-tacgactcactataggg-3'
Dim2-HTPF	5'-AAGTTTATGGGAACTTACGTACCGTTGCATCTAGATTAAAAGAACGCTAC-gagcaccatcaccatcacc-3'
Dim2-HTPR	5'-TAAAACGACATATAAATATTATACAGATGATGAAAGCCCACAAATTATGT-tacgactcactataggg-3'
Tsr1-HTPF	5'-CCTTGTACAAACGTATGTGGCCCATGCCTTCGTTACCTTGGAATGGTATG-gagcaccatcaccatcacc-3'
Tsr1-HTPR	5'-GTATCGTTGATACTATTTTATTAGCATTATATTATACAAATAGATCTCAC-tacgactcactataggg-3'
Enp1-HTPF	5'-AGTTTGTTGATCCACAGGAAGCTAATGATGATTTAATGATTGATGTCAAT-gagcaccatcaccatcacc-3'
Enp1-TAPR	5'-GGGGGAAAGACCGAGCGATATAAAATTGATGAAAAATTGATATTACAGCA-tacgactcactataggg-3'
Ltv1-HTPF	5'-AGAAAAAGAAACTTGAGAAGGTCACCAATACACTAAGCAGCTTAAAATTT -gagcaccatcaccatcacc-3'
Ltv1-TAPR	5'-GTCTACACAGTACTTGTAATGTAGGTGCTTTCTCATCTCATTCTACTCCT-tacgactcactataggg-3'
004	5'-CGGTTTTAATTGTCCTA-3'
033	5'-CGCTGCTCACCAATGG-3'
020	5'-TGAGAAGGAAATGACGCT-3'
ITS1 RT	5'-CCATCTCTTGTCTTCTTGCCCAG-3'

Supplementary Table 2: Oligonucleotides used in this study.

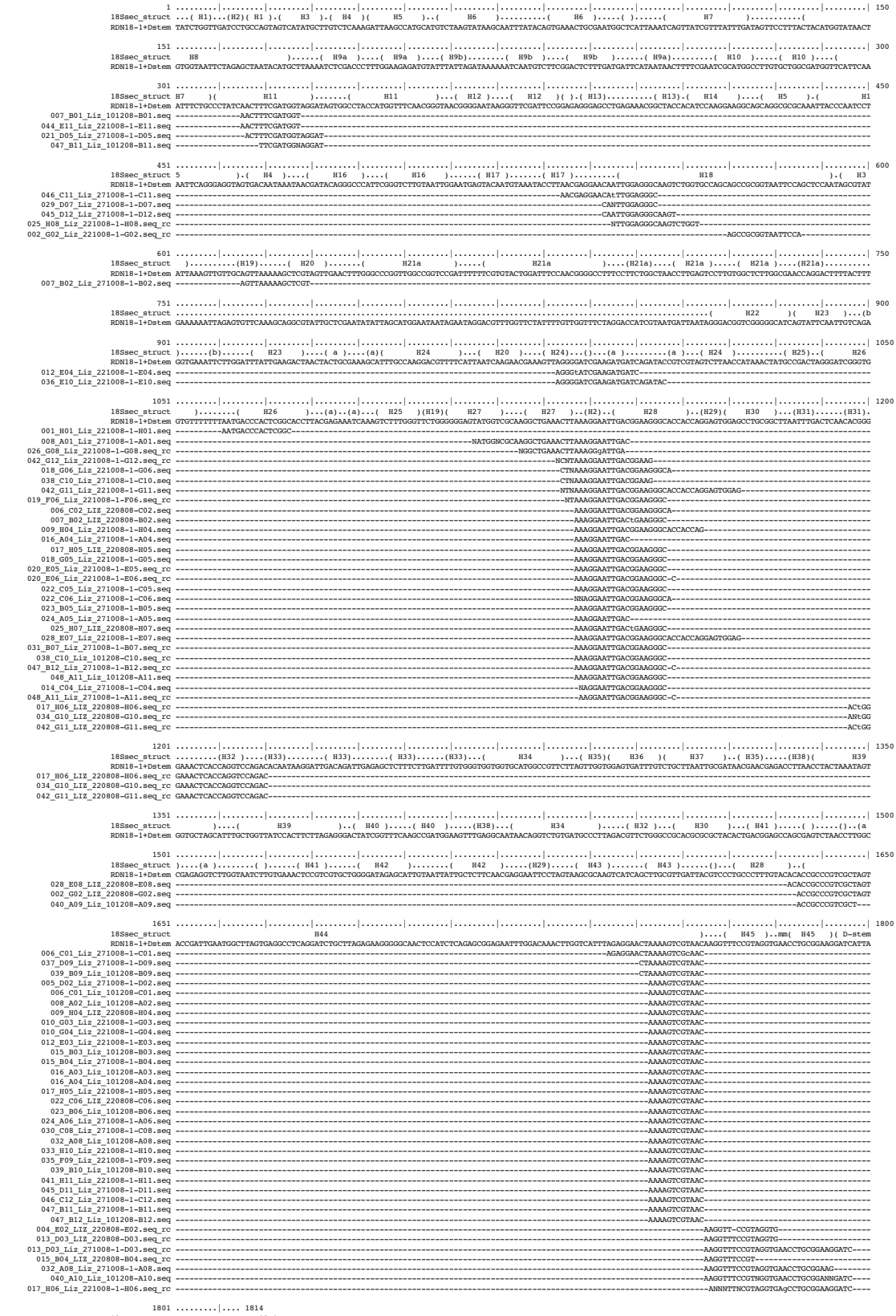
Lowercase sequences indicate the plasmid specific sequences.

Strain	Genotype	Reference
BY4741	MATa; *his3Δ1; leu2Δ0; met15Δ0; ura3Δ0*	(Brachmann et al, 1998)
D1076	as BY4741 but with *dim1*-HTP*::K.l.URA3*	This study
D1084	as BY4741 but with *dim2*-HTP*::K.l.URA3*	This study
D1092	as BY4741 but with *enp1*-HTP*::K.l.URA3*	This study
D1091	as BY4741 but with *nob1*-HTP*::K.l.URA3*	This study
D1077	as BY4741 but with *rio1*-HTP*::K.l.URA3*	This study
D1079	as BY4741 but with *rio2*-HTP*::K.l.URA3*	This study
D1081	as BY4741 but with *ltv1*-HTP::K.l.URA3	This study
D1089	as BY4741 but with *tsr1*-HTP::K.l.URA3	This study
YAF34	MATa ade2-1 his3-11, 15 leu2-3, 112 trp1- ura3-1 KAN::GAL::HA-*nob1*),	(Fatica et al, 2003)

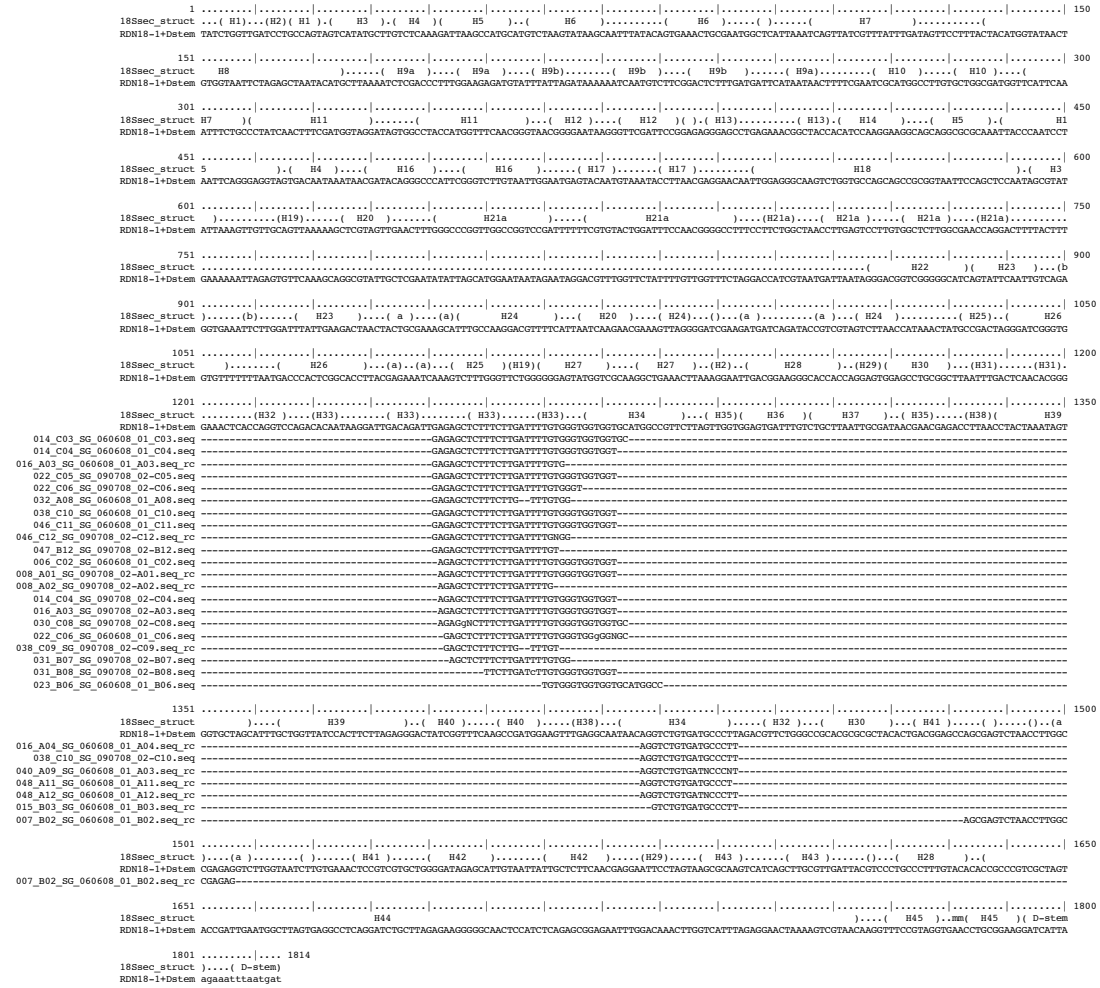
Supplementary Table 3: Yeast strains used in this study.

Supplementary Tables 4-9

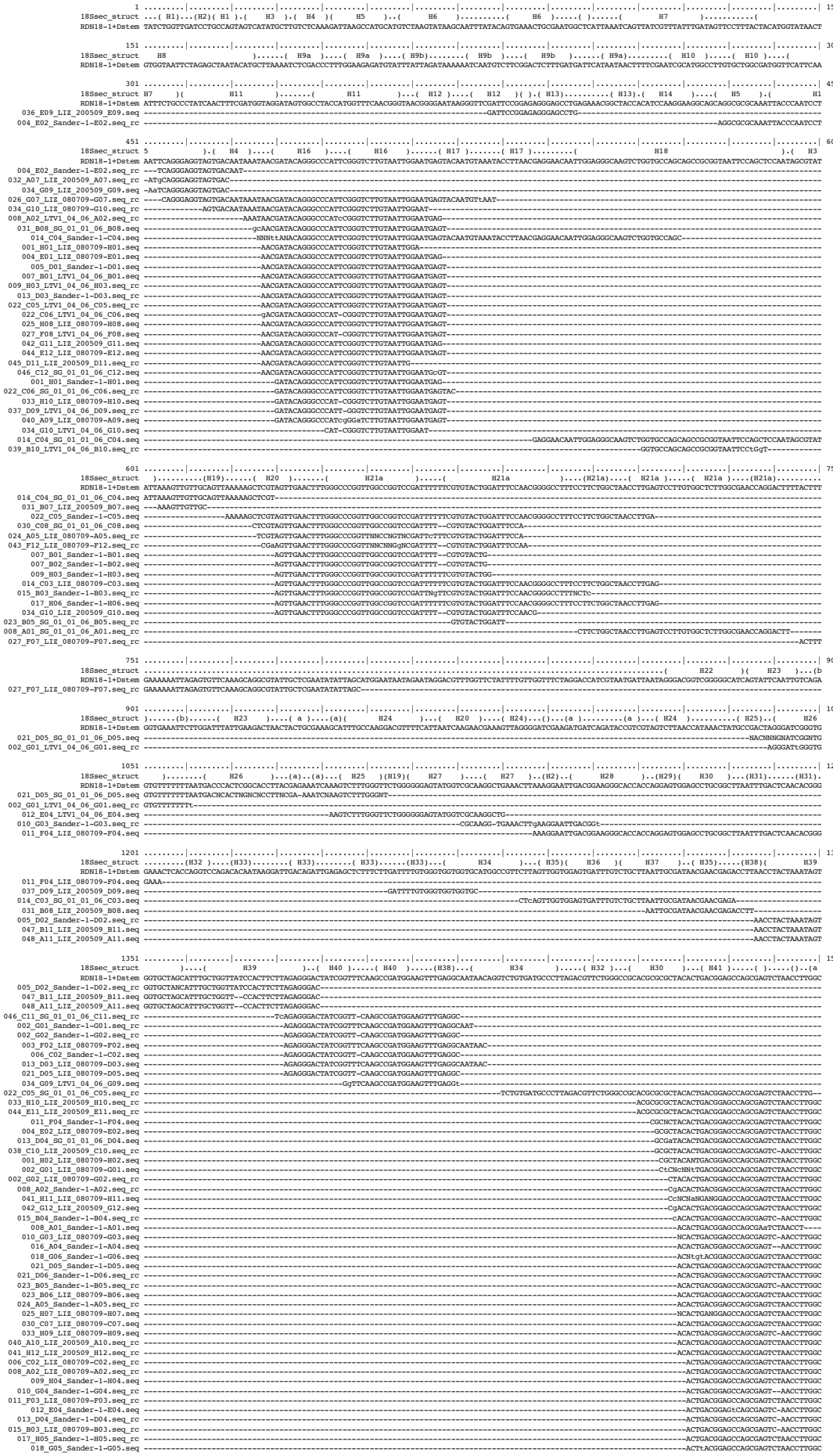
Multiple sequence alignments: Sequences from 2-5 independent experiments were aligned to the rDNA sequence using Novoalign 2.05 (www.novocraft.com). Using various Perl scripts, we then converted the Novoalign output to a multiple sequence alignment. Lowercase letters indicate nucleotide subsitutions. Dashes between nucleotides indicate deletions. Mutations were then used to pinpoint the protein cross-linking site(s). The second row in each file indicates the secondary structure of the 18S rRNA. A 'dot' indicates single stranded nucleotides, whereas parentheses indicate nucleotides that are base-paired. The secondary structure information was obtained from http://www.rna.ccbb.utexas.edu/.

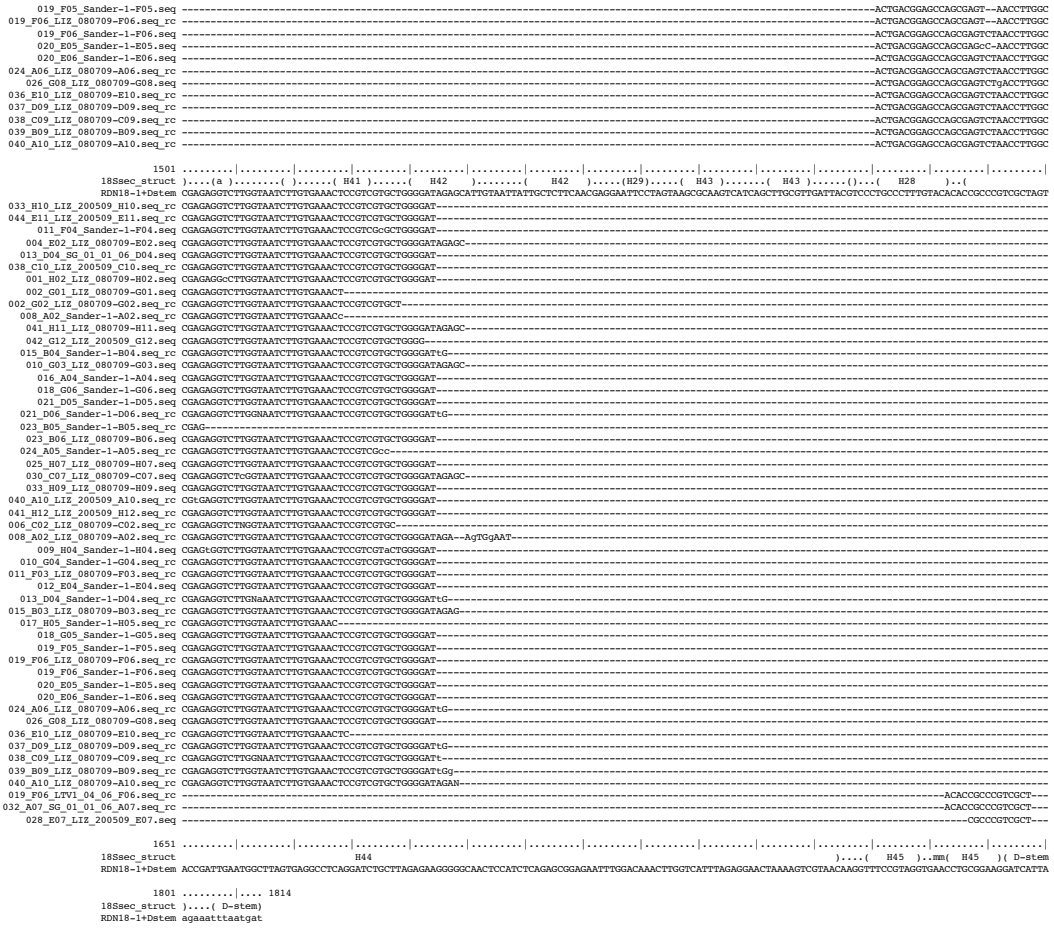


Granneman Supplementary Table 4: Hits obtained for Dim1 aligned against 18S rRNA

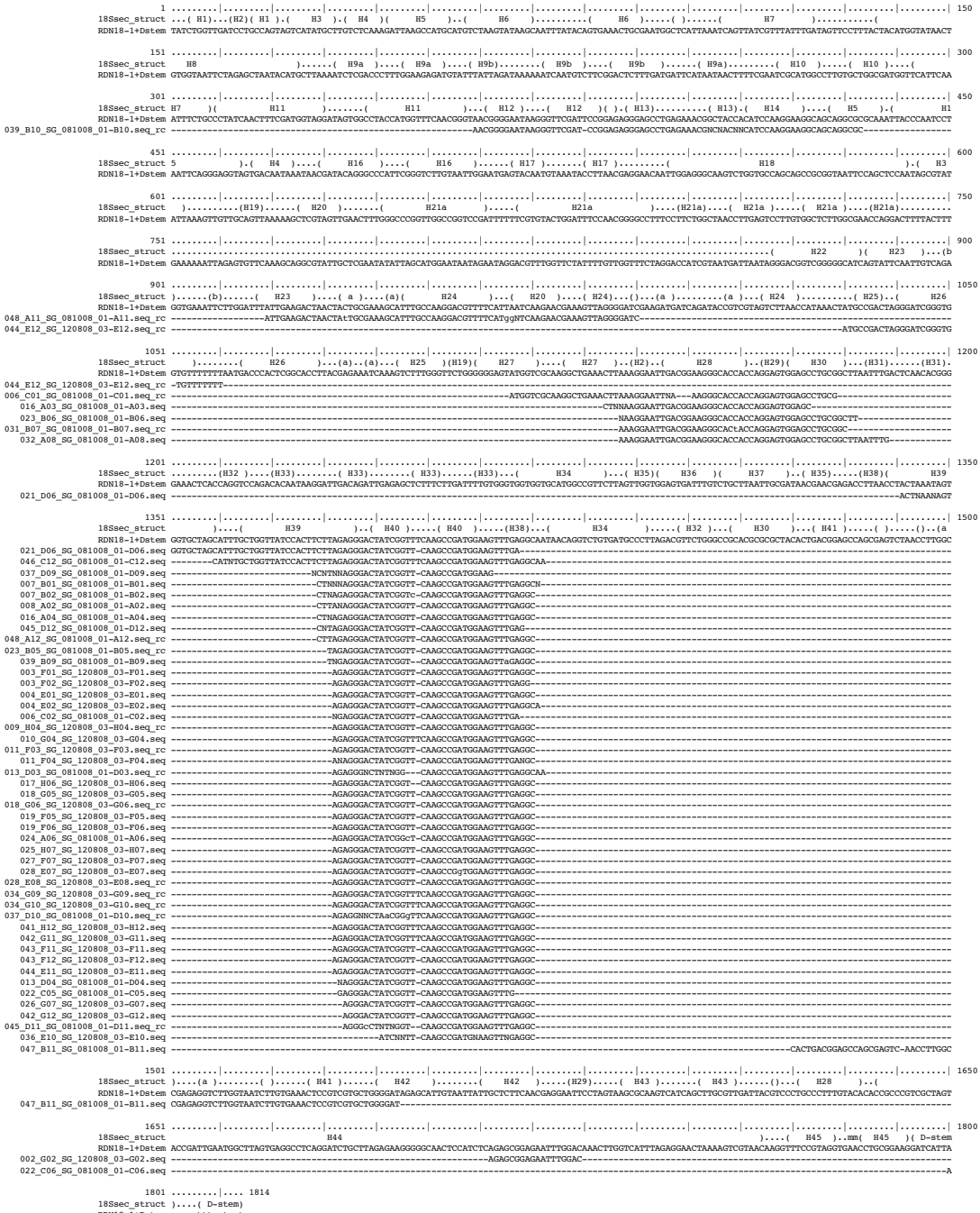


Granneman Supplementary Table 5: Hits obtained for Enp1 aligned against 18S rRNA:

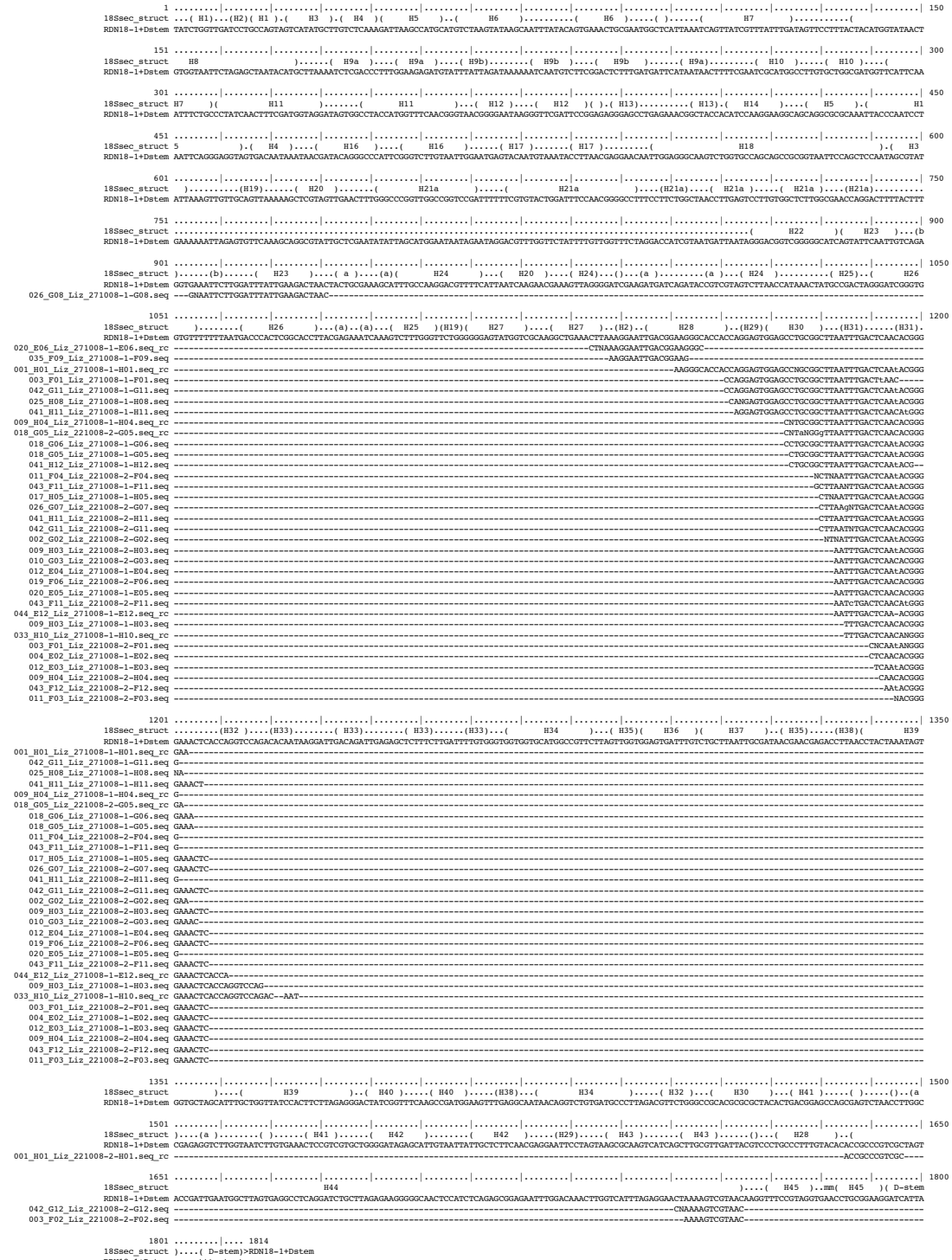




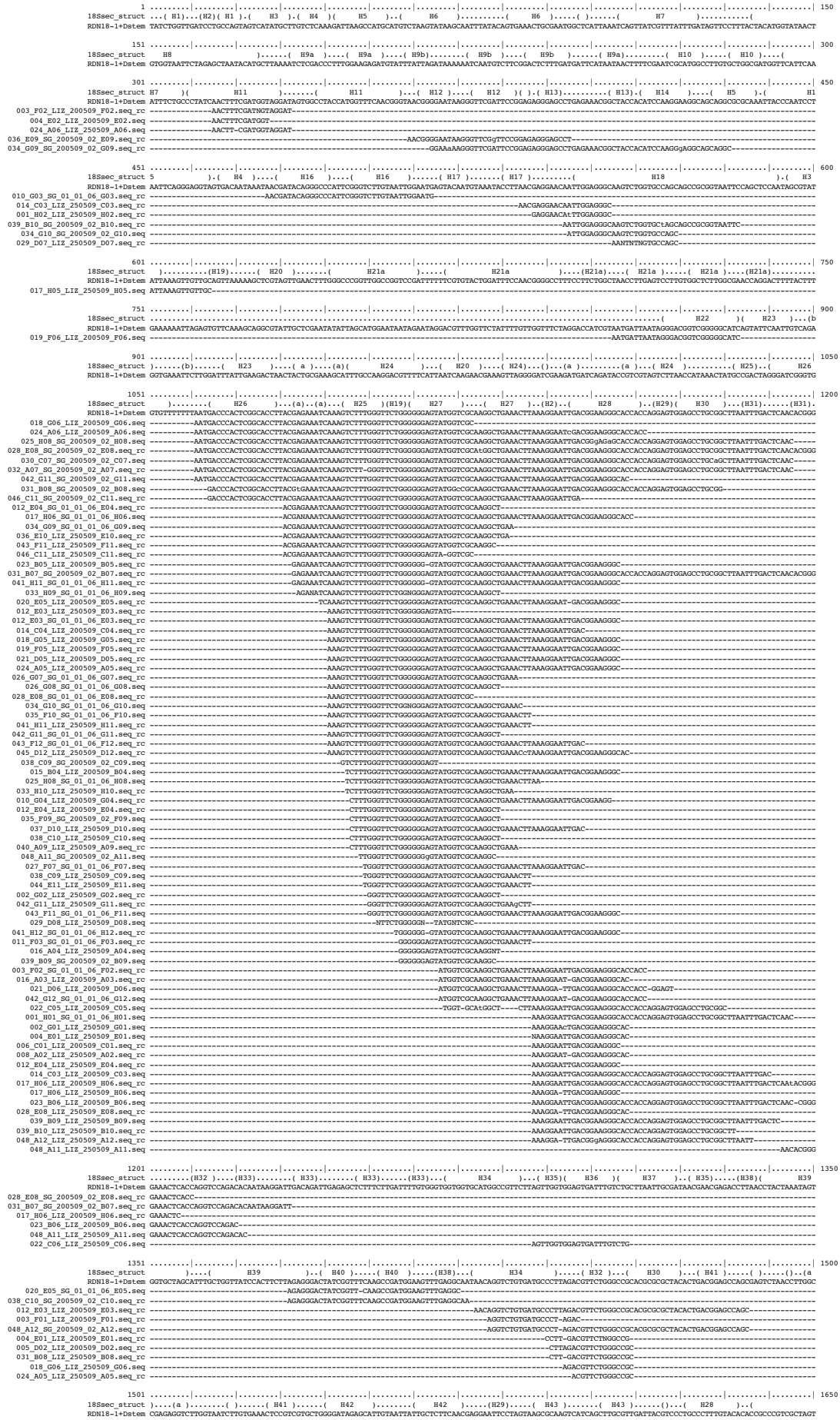
Granneman Supplementary Table 6: Hits obtained for Ltv1 aligned against 18S rRNA

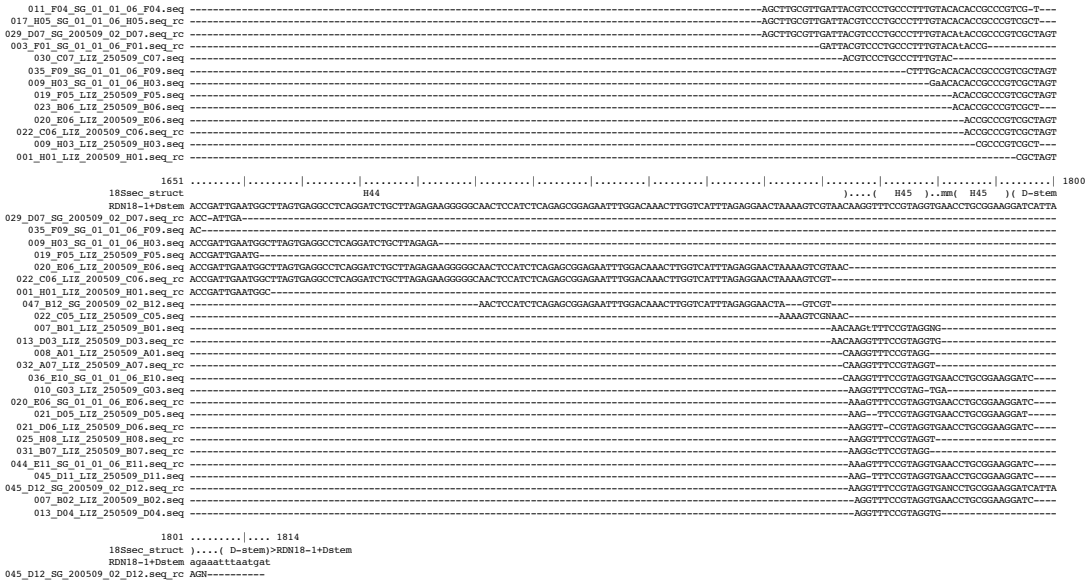


Granneman Supplementary Table 7: Hits obtained for Nob1 aligned against 18S rRNA

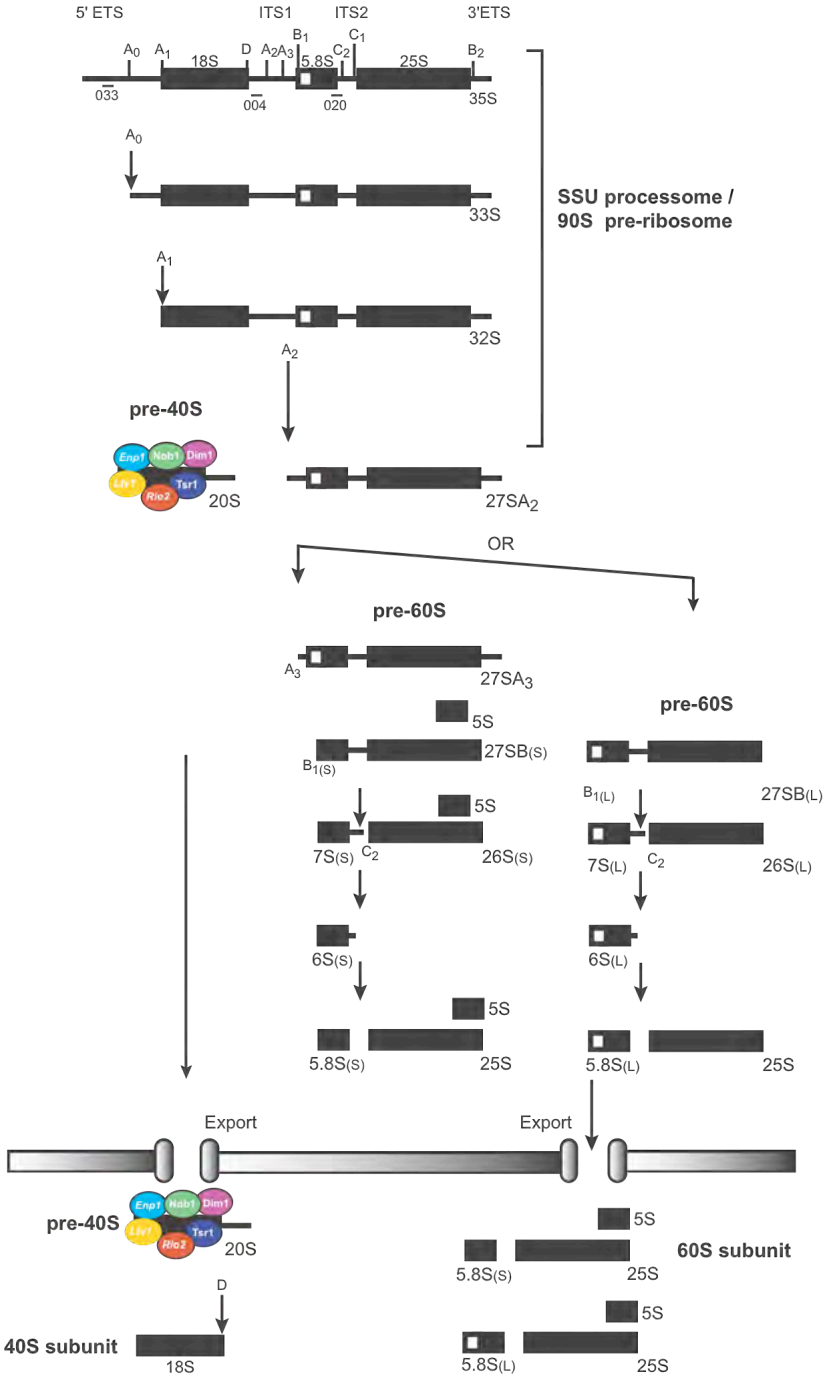


Granneman Supplementary Table 8: Hits obtained for Rio2 aligned against 18S rRNA





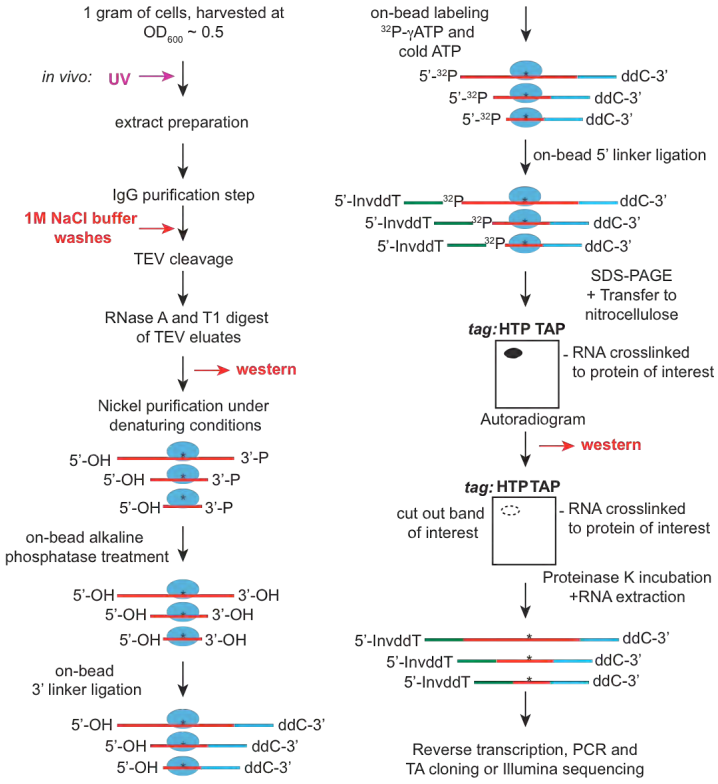
Granneman Supplementary Table 9: Hits obtained for Tsr1 aligned against 18S rRNA



Granneman et al 2010 Supplementary Figure 1

Supplementary Figure 1. Pre-rRNA processing in *Saccharomyces cerevisiae*

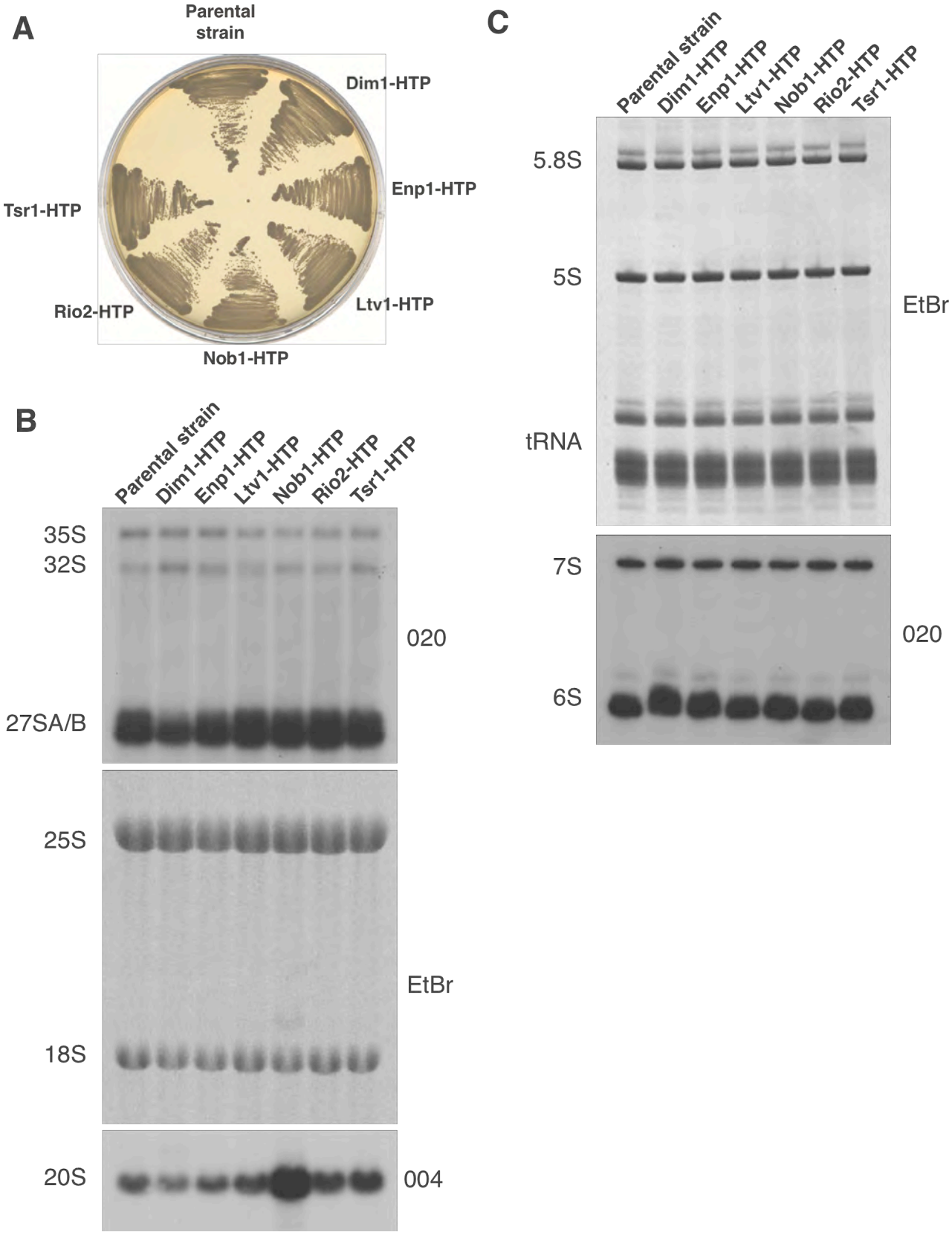
Shown is a schematic representation of pre-rRNA processing in yeast. The locations of processing sites on the 35S pre-rRNA are indicated. In the nucleolus, the 35S pre-rRNA, part of a 90S-sized complex called the SSU processome or 90S pre-ribosome, is processed at sites A_0, A_1 and A_2, leading to the formation of 43S and 66S pre-ribosomes. Proteins studied in the CRAC analysis and their association with pre-ribosomal complexes is indicated. The 43S pre-ribosome is exported to the cytoplasm where Nob1 cleaves at site D, yielding the mature 18S rRNA and 40S subunit. 66S pre-ribosomes containing $27SA_2$ pre-rRNA are either processed at A_3 or at $B1_{(L)}$. Pre-ribosomes containing $27SA_3$ pre-rRNA are exonucleolytically trimmed, yielding the $27SB_{(S)}$ pre-rRNA. After this step, the 27SB is cleaved at C_2 by an unknown endonuclease, followed by exo-and endo-nucleolytic trimming of 7S by the exosome.



Granneman et al 2010 Supplementary Figure 2

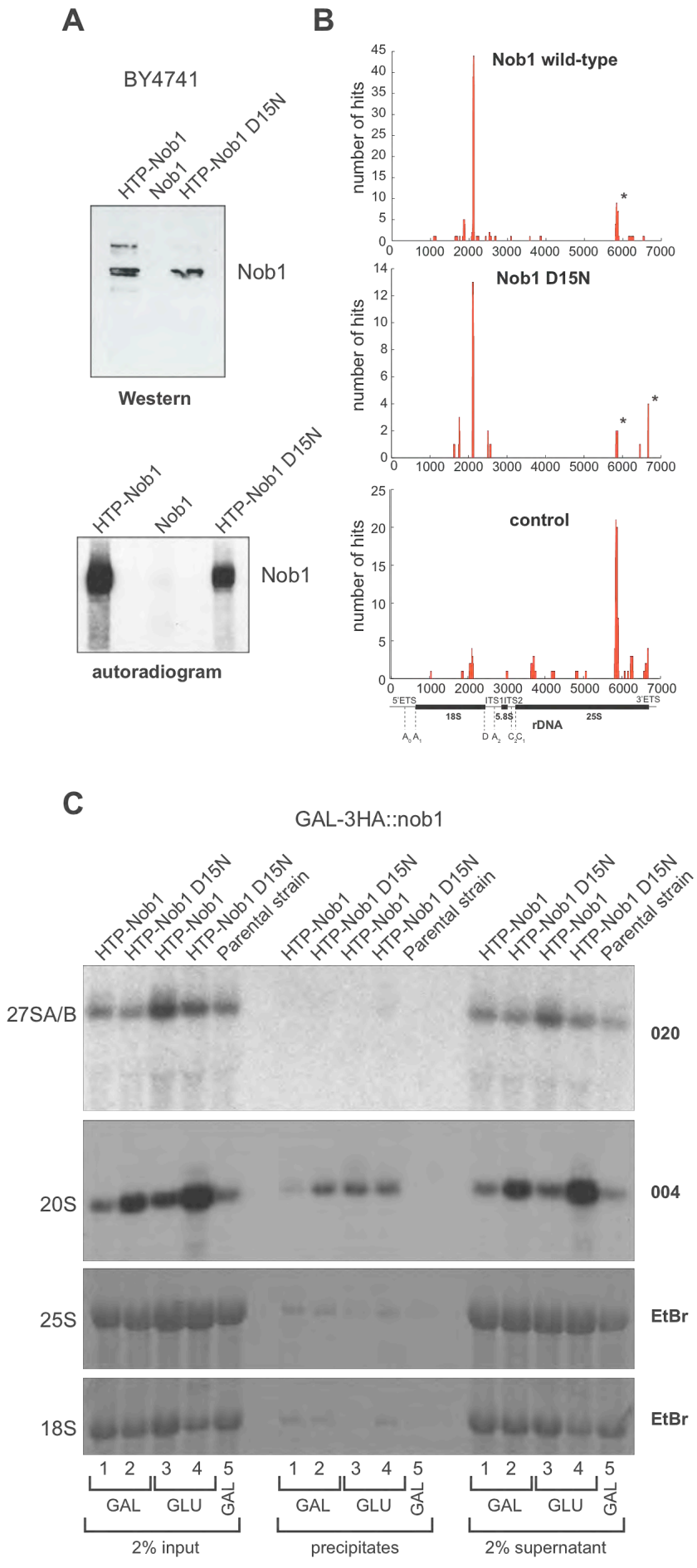
Supplementary Figure 2. Overview of the CRAC method.

Schematic overview of the UV cross-linking and cloning method (CRAC). Cells expressing 2xProtA-TEV-His_6(HTP)-tagged proteins were UV-irradiated in petri dishes on ice. Extracts were incubated with IgG beads and after extensively washing the beads with a buffer containing 1M NaCl, tagged proteins were released by TEV protease cleavage. Five percent of the TEV eluate was analyzed by Western blotting. Partially RNase digested RNPs were denatured with Guanidine (6M final) and incubated with nickel beads to immobilize His_6-tagged proteins (blue ovals) and covalently attached RNAs (red lines). Cross-linked RNAs were 3' dephosphorylated, ligated to the adenylated linker (blue line), radioactively labeled with polynucleotide kinase and then ligated to the 5' linker (green line). Following release by imidazole treatment, radioactive RNPs were resolved on Bis-Tris NuPAGE gels and transferred to nitrocellulose. Bands corresponding to the predicted *Mr* of the target protein were excised, digested with proteinase K and recovered RNAs were amplified by RT/PCR. The PCR products were gel-purified and sequenced. ddC: dideoxy-cytidine. InvddT: inverted dideoxythymidine. The asterisk indicates the UV cross-linking site.



Granneman et al 2010 Supplementary Figure 3

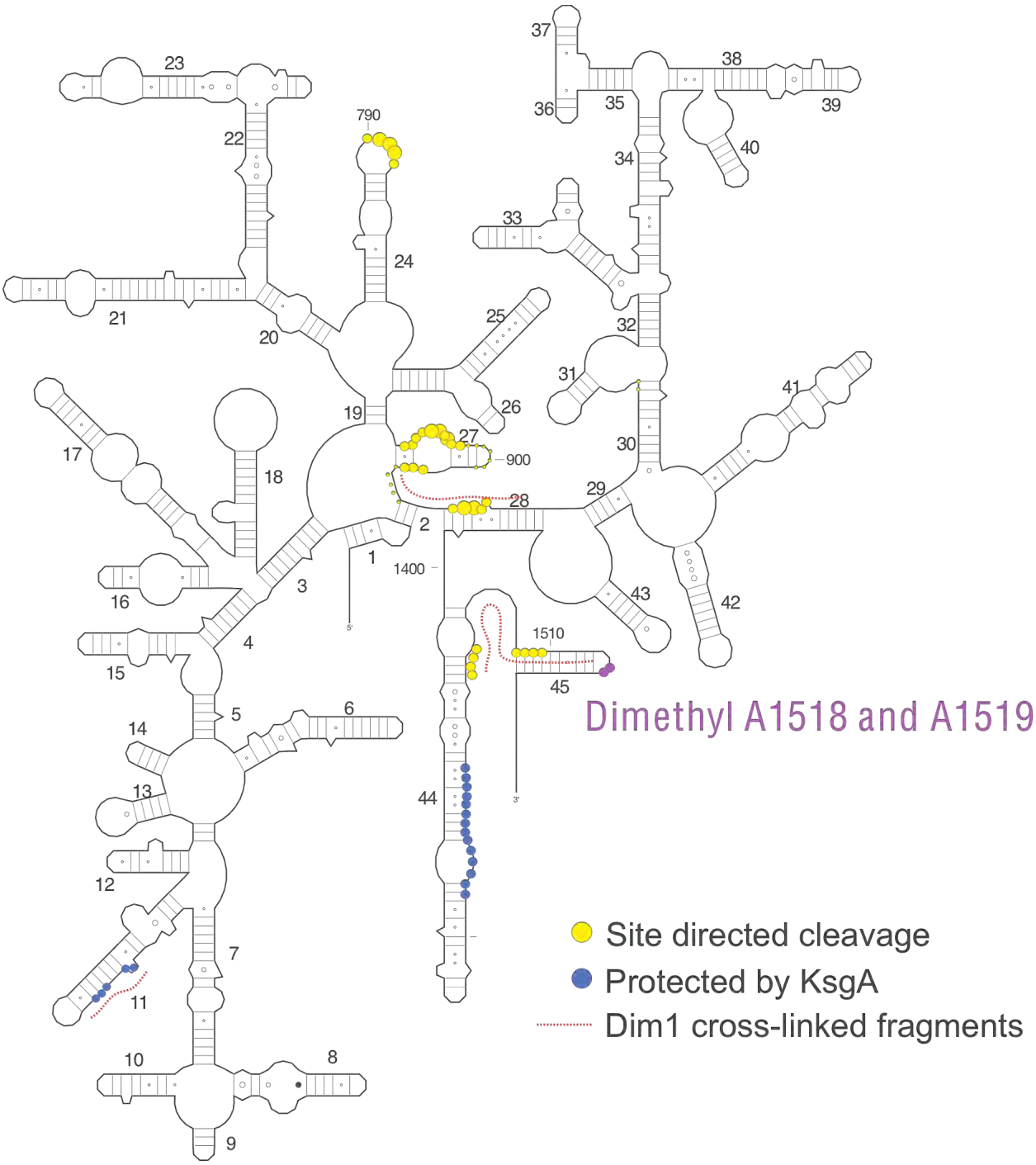
Supplementary Figure 3. Effect of C-terminal HTP tag on cell growth and protein function. (**A**) The HTP-tagged strains do not show any noticeable growth defect. HTP-tagged proteins were streaked on YPD and grown at 30ºC for two days. (**B**-**C**) Pre-rRNA processsing in HTP-tagged strains. Total RNA was extracted from exponentially growing cells and 4 μg was resolved on a 1.2% agarose gel (**B**) and 8% Polyacrylamide/8M Urea gel. RNA was visualized by staining the gel with ethidium bromide (EtBr, 25S, 18S, 5S and 5.8S) or by Northern blot analysis on Hybond N^+ membranes using radiolabeled oligonucleotides 004 (20S, 23S) and 020 (35S, 27S, 7S pre-rRNAs).



Granneman et al 2010 Supplementary Figure 4

Supplementary Figure 4. The active site of the Nob1 PIN domain is not involved in binding to helix 40 in the 18S rRNA

(**A**) Nob1 PIN domain mutant still cross-links to RNA. The BY4741 parental strain (Nob1), BY4741 expressing an N-terminally HTP-tagged Nob1 (HTP-Nob1) and BY4741 cells expressing the HTP-tagged Nob1 PIN mutant (HTP-Nob1 $D_{15}N$) were grown in 1L of minimal media without leucine to an OD_{600} of 0.5. *In vivo* CRAC was subsequently performed as described previously (Granneman et al, 2009). Proteins in Nickel eluates were detected using anti His_6-HRP antibodies from Sigma as described (Granneman et al, 2009). Cross-linking of Nob1 proteins was performed as outlined in Supplementary Figure 2. Cross-linked RNAs were detected by autoradiography. (**B**) Nob1 PIN mutant still cross-links to helix 40. Plotted is the number of times a nucleotide in an RNA fragment was mapped to the rDNA reference sequence (schematically represented below the x-axis of the control experiment histogram). Cleavage sites in spacer regions are indicated with dashed lines. (**C**) HTP-tagged Nob1 and Nob1-$D_{15}N$ preferentially co-precipitates the 20S pre-rRNA. Immunoprecipitations were performed with the GAL::3HA-*nob1* strain grown in minimal medium without leucine containing galactose as carbon source or for 8 hours in glucose containing medium lacking leucine, as indicated. Cells harbored an empty vector (pRS415, lanes 5, 10 and 15), expressed the wild-type N-terminally tagged HTP-Nob1 proteins (lanes 1, 3, 6, 8, 11 and 13) or expressed the HTP-Nob1 $D_{15}N$ mutant (lanes 2, 4, 7, 9, 12 and 14). To control for RNA loading and quality of the RNA, two percent of input and supernatant after immunoprecipitation was extracted. RNAs were resolved on 1.2% agarose gels, transferred to Hybond N+ (GE healthcare) and probed with radiolabeled oligonucleotides 004 (20S, 23S) and 020 (27SA/B pre-rRNAs). To visualize the mature 18S and 25S rRNAs, the agarose gel was stained with ethidium bromide (EtBr).



Granneman et al 2010 Supplementary Figure 5

Supplementary Figure 5. Dim1 cross-links to rRNA at positions analogous to the KsgA 16S rRNA interaction sites.

Shown is a schematic representation of the *E. coli* 16S rRNA secondary structure. The yellow spheres indicate KsgA interaction sites on the 16S rRNA identified by directed hydroxyl radical probing using an Fe(II)-modified KsgA bound to the 30S subunit. Blue spheres indicate nucleotides protected from hydroxyl radical cleavage by KsgA on the 30S subunit (figure modified with permission (Xu et al, 2008)). Cleavage sites were identified by primer extension and the sphere size indicates signal intensity of the primer extension stop (Xu et al, 2008). The purple spheres indicate the KsgA methylation sites. The red dashed lines indicate the positions of the yeast Dim1 18S rRNA binding sites.

References

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