**The Bowen-Conradi syndrome protein Nep1 (Emg1) has a dual role in eukaryotic ribosome biogenesis, as an essential assembly factor and in the methylation of Ψ1191 in yeast 18S rRNA**

**Supplementary Data**

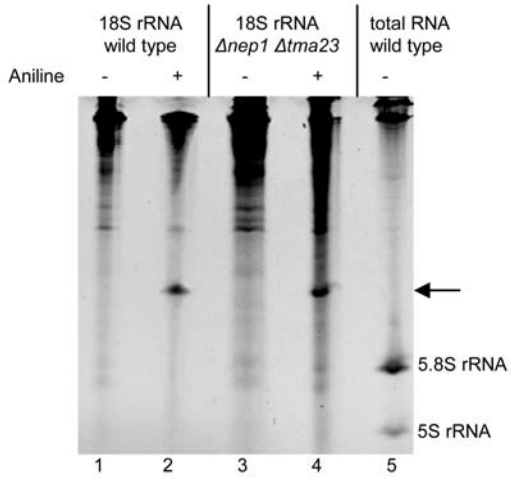



**Figure S1. Analysis of the G1575 yeast 18S rRNA modification**

$m^7G$ (N7-methyl-guanosine) aniline cleavage of 18S rRNA from wild type (lane 2) and a *Scnep1* Δ*tma23* strain (lane 4). Lanes 1 and 3 correspond to the 18S rRNAs of wild type and the *Scnep1* Δ*tma23* strain without aniline treatment. Lane 5 corresponds to total RNA from wild type. The position of the 3' terminal 18S rRNA cleavage product (nt 1576-1800) is marked.

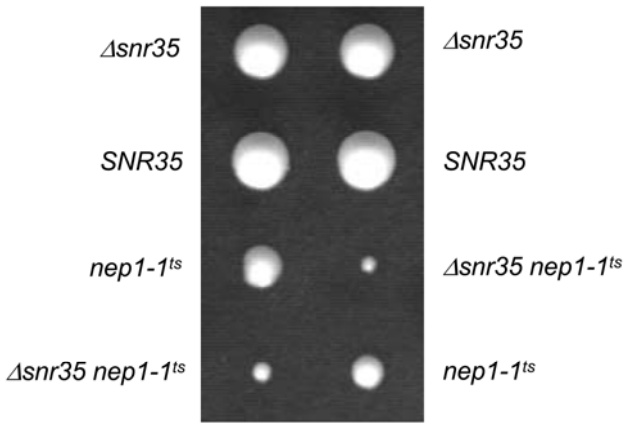



**Figure S2. Recombinant analysis of *ScΔsnr35* and *nep1-1$^{ts}$* mutations**

Segregant colonies of tetrads obtained after sporulation of the diploids (*ScΔsnr35/SNR35 Scnep1-1$^{ts}$/NEP1*) were analyzed after 3 days of growth at 23°C.

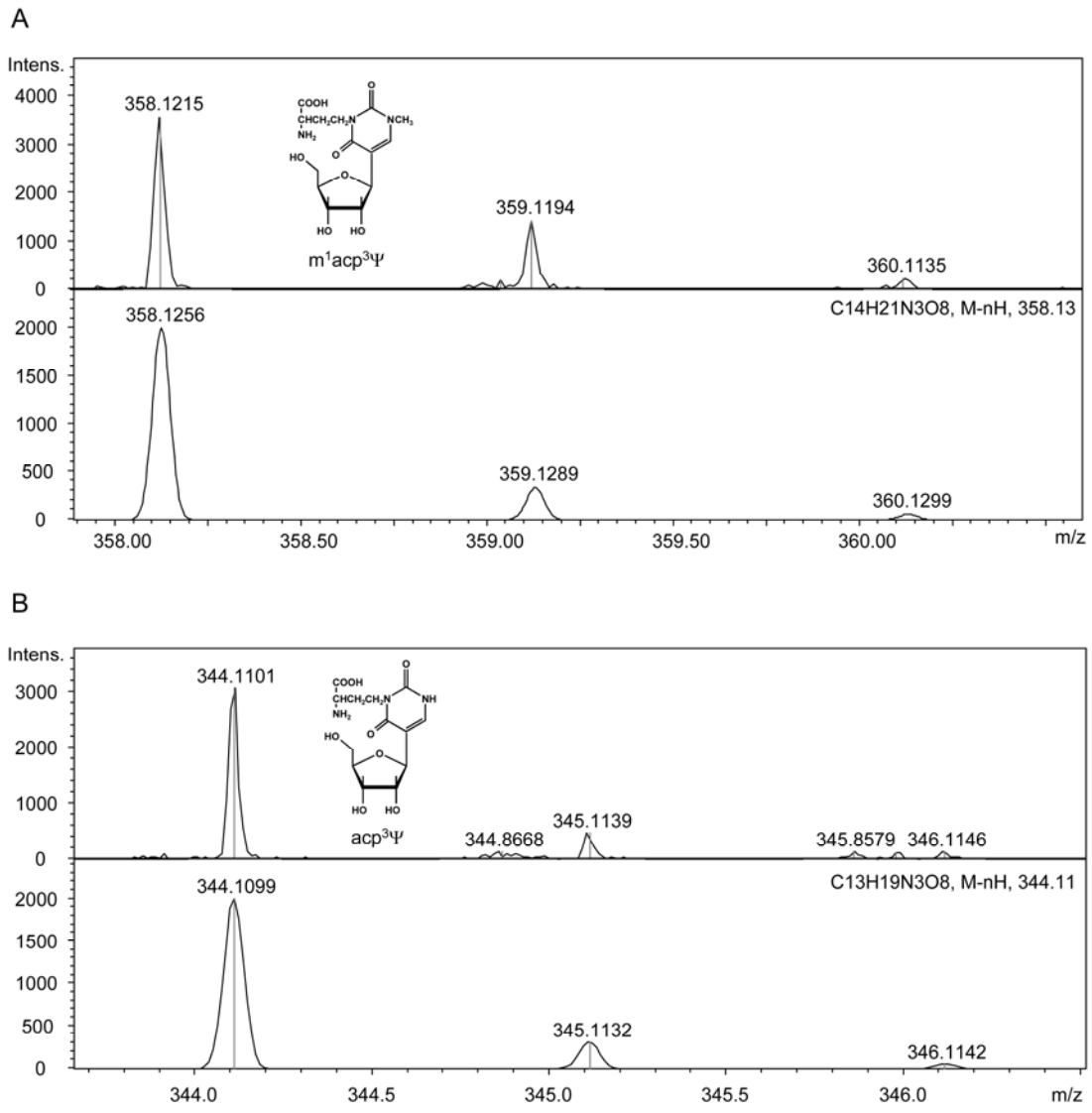
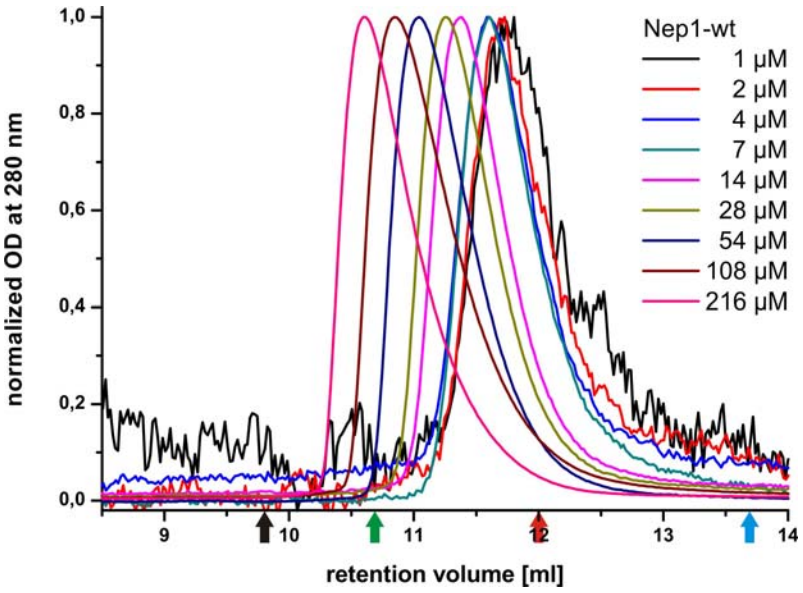



**Figure S3. Analysis of U1191 modification in wild type and *ScΔnep1 Δnop6* cells**

**(A)** ESI mass spectrometry analysis of the m1acp3Ψ peak from wild type (see also figure 2B). Upper lane corresponds to the measured ESI-MS spectrum, lower lane corresponds to the theoretically expected spectrum.

**(B)** ESI mass spectrometry analysis of the acp3Ψ peak from the *ScΔnep1 Δnop6* mutant (see also figure 2B). Upper lane corresponds to the measured ESI-MS spectrum, lower lane corresponds to the theoretically expected spectrum.

A




B

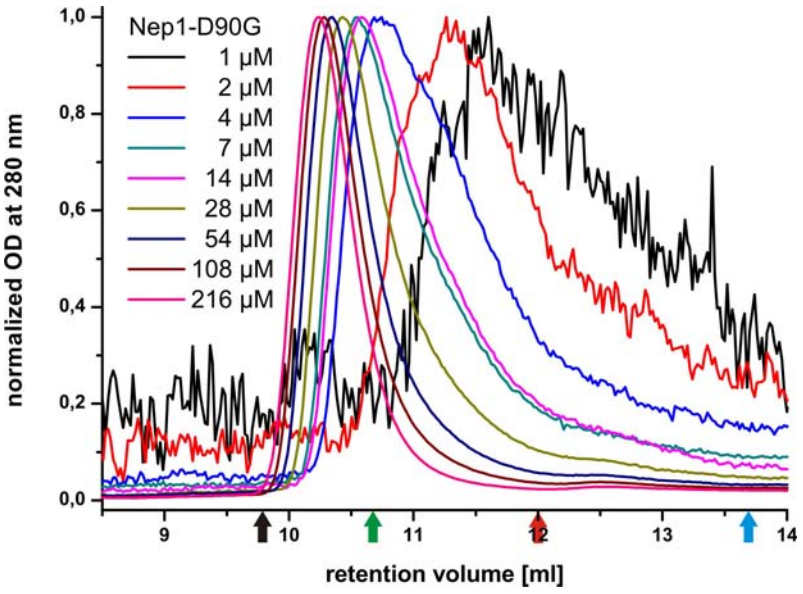



**Figure S4: Overlay of gel filtration chromatograms at different protein concentrations.** Overlay of the normalized gel filtration chromatograms of wild type *Sc*Nep1 **(A)** and *Sc*Nep1$^{D90G}$ **(B)** at different concentrations (1-216 µM). The elution volumes of the molecular mass standards are indicated by arrows (black, conalbumin: 75 kDa, green, ovalbumin: 43 kDa, red, carbonic anhydrase: 29 kDa, blue, ribonuclease A: 13.7 kDa).

## Supplementary Methods

### Plasmid constructions

For cellular localization of GFP-fusion proteins the *ScNEP1* open reading frame was amplified and ligated into plasmid pUG34 (kindly provided by H. Hegemann) as previously described (1) The D90G point mutation was introduced into plasmids pUG34-Nep1 and pGAD-Nep1 by site-directed mutagenesis with primers NEPD90G-1 and NEPD90G-2. Yeast *ScNEP1* wild type and *ScNEP1*$^{D90G}$ mutant genes were amplified with primers NEP1-NsiI and NEP1-BamHI-I (see Table S2 for Oligonucleotides) and cloned into pQE-9 (QIAGEN) cleaved with *Pst*I and *Bam*HI. Plasmid pAV164 (kindly provided by S. Liebman) carries the yeast wild-type 9 kb rDNA unit and served as source of yeast rDNA. For *in vitro* mutagenesis a part of the rDNA unit was first subcloned. A 2675 bp *Sac*II/*Mlu*I fragment from pAV164 containing parts of the 18S rDNA from nucleotide 576 to 1800 were inserted in plasmid pUCBM20 (Boehringer Mannheim) opened with the same restriction enzymes, resulting in plasmid pPK481. Mutations in the 18S rDNA were introduced into pPK481 by site-directed mutagenesis using primers 18S-Mut3/18S-Mut4 resulting in plasmid pPK495 (U1191 to C) and pPK497 (U1191 to A), resp. The mutagenized *Sac*II/*Mlu*I fragment from pPK495 and pPK497, resp., was inserted in pAV164 by replacing the wild-type *Sac*II/*Mlu*I fragment resulting in plasmids pPK499 (U1191 to C) and pPK501 (U1191 to A), resp. All plasmids were verified by sequencing.

### Yeast strain constructions

All strains used in this study (see Table S1 for Yeast strains) except L40-coat and Nop56-mRFP are isogenic to CEN.PK2 (2). For PCR mediated deletion of open reading frames the lox-KanMX4-loxP gene disruption cassette was amplified with the appropriate primers and plasmid pUG6 as template (3). For amplification of the *ScSNR35* deletion cassette primers SNR35-2del5 and SNR35-2del3 were used, for the deletion of *ScMET13* primers MET13-S1 and MET13-S2. PCR mediated deletion of *ScTMA23* and *ScNOP6* was described previously (4). All double and triple mutants were constructed by mating of single mutants and sporulation of resulting diploids followed by tetrad dissection. For conversion of selectable markers in the yeast genome “marker swap plasmids” were used (5).

The *ScNEP1*$^{D90G}$ point mutation (GAT → GGT) was introduced into the genome with the “delitto perfetto” method (6). Therefore the disruption cassette was amplified with primers

Core-NEP-5 and Core-NEP-3 and integrated into the *ScNEP1* open reading frame (downstream of nucleotide 269) of a haploid strain which contained the human *HsNEP1* cDNA on a multicopy plasmid. For replacement of the marker cassette and introduction of the point mutation dsDNA obtained after hybridization of oligonucleotides D90G-e and D90G-f was transformed into the *Scnep1*-disrupted strain. 5-FOA resistant transformants with the *ScNEP1*$^{D90G}$ mutation were isolated and tested for their ability to loose the human *HsNep1* encoding plasmid. For creation of strains with *ScNEP1* under translational control of the tc aptamer, an insertion cassette containing *ScTDH3* promotor, 3 tc aptamers and a 3xHA epitope was amplified from plasmid pTDH3p-tc3-3xHA (7) with primers NEP1-Tc-1 and NEP1-Tc-2 and integrated upstream of the *ScNEP1* open reading frame in the wild type (CEN.PK2-1C) or the *ScNEP1*$^{D90G}$ mutant CEN.BM52-1C.

Complete deletion of yeast chromosomal rDNA repeats in CEN.PK strains were obtained as described previously (8). CEN.PK2-1C was transformed with plasmid pRDN-hyg1 (high copy *URA3*, kindly provided by S. Liebman) and the stable hygromycin-resistent strain CEN.PK912-1C was isolated in which most rDNA repeats were deleted. To remove the remaining rDNA repeats, CEN.PK912-1C was transformed with the linear *Bam*HI cleaved plasmid pNOY455 (kindly provided by M. Nomura) resulting in strain CEN.PK920-1C with a complete chromosomal rDNA deletion and plasmid pRDN-hyg1. To remove the pRDN-hyg1 plasmid, strain CEN.PK920-1C was transformed with high copy plasmids (*TRP1 LEU2*$^{d}$) carrying wild type rDNA (pAV164) or mutant rDNA (pPK499 and pPK501), respectively. Strains were cured of plasmid pRDN-hyg1 by selection on 5-FOA medium, finally resulting in strains CEN.PK968-1C (wild type), CEN.PK963-1C (U1191C) and CEN.PK966-1C (U1191A), resp. All strains were verified by Southern analysis.

**Analysis of G1575 N7-methylation**

Specific aniline-mediated fragmentation of 18S rRNA at N7-methylguanosine 1575 was carried out as previously described (9) and resulting RNA-fragments were separated on a denaturing 8 % acrylamide gel.

## Table S1. Yeast strains

| Strain | Genotype | Origin |
|---|---|---|
| CEN.PK2 | *MATa/MATα ura3-52/ura3-52 trp1-289/trp1-289 leu2-3,112/leu2-3,112 his3 Δ1/his3-Δ1 MAL2-8$^C$/MAL2-8$^C$ SUC2/SUC2* | (2) |
| CEN.PK2-1C | *MATa ura3-52 trp1-289 leu2-3,112 his3-Δ1 MAL2-8$^C$ SUC2* | (2) |
| CEN.PK2-1D | *MATα ura3-52 trp1-289 leu2-3,112 his3-Δ1 MAL2-8$^C$ SUC2* | (2) |
| CEN.SR679 | *MATa/MATα ura3-52/ura3-52 trp1-289/trp1-289 leu2-3,112/leu2-3,112 his3 Δ1/his3-Δ1 MAL2-8$^C$/MAL2-8$^C$ SUC2/SUC2 NEP1/Δnep1::loxP-KanMX4-loxP* | (1) |
| CEN.SR679-1A-ts1 | *MATα ura3-52 trp1-289 leu2-3,112 his3-Δ1 MAL2-8$^C$ SUC2 nep1-1$^{ts}$* | (1) |
| CEN.MB9-3A | *MATα ura3-52 trp1-289 leu2-3,112 his3-Δ1 MAL2-8$^C$ SUC2 Δnep1::loxP-KanMX4-loxP Δtma23::loxP-KanMX4-loxP* | (4) |
| Nop56-mRFP | *Mat α his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 met15Δ0 NOP56-mRFP-KanMX6* | (10) |
| CEN.NM1-4D | *MATa URA3 TRP1 LEU2 HIS3 MAL2-8$^C$ SUC2 Δmet13(41,1760)::loxP-KanMX4-loxP* | This work |
| CEN.BM140-11B | *MATa leu2-3,112 TRP1 URA3 his3-Δ1 MAL2-8$^C$ SUC2 Δnep1::loxP-KanMX4-loxP Δnop6::loxP-LEU2-loxP Δmet13(41,1760)::loxP-KanMX4-loxP* | This work |
| CEN.BM141-7G | *MATa TRP1 LEU2 URA3 his3-Δ1 MAL2-8$^C$ SUC2 met13(41,1760)::loxP-KanMX4-loxP Δsnr35::loxP-kanMX4-loxP* | This work |
| CEN.PK968-1C | *MATa ura3-52 trp1-289 leu2-3,112 his3-Δ1 MAL2-8$^C$ SUC2 ΔΔrdn::pNOY455 + pAV164* | This work |
| CEN.PK963-1C | *MATa ura3-52 trp1-289 leu2-3,112 his3-Δ1 MAL2-8$^C$ SUC2 ΔΔrdn::pNOY455 + pPK499 (1-5)* | This work |
| CEN.PK966-1C | *MATa ura3-52 trp1-289 leu2-3,112 his3-Δ1 MAL2-8$^C$ SUC2 ΔΔrdn::pNOY455 + pPK501 (1-4)* | This work |
| CEN.BM146-1C | *MATa ura3-52 trp1-289 leu2-3,112 his3-Δ1 MAL2-8$^C$ SUC2 Δmet13(41,1760)::loxP-KanMX4-loxP ΔΔrdn::pNOY455 +* pAV164 | This work |
| CEN.BM147-1C | *MATa ura3-52 trp1-289 leu2-3,112 his3-Δ1 MAL2-8$^C$ SUC2 Δmet13(41,1760)::loxP-KanMX4-loxP ΔΔrdn::pNOY455 +* pPK499 (1-5) | This work |
| CEN.BM148-1C | *MATa ura3-52 trp1-289 leu2-3,112 his3-Δ1 MAL2-8$^C$ SUC2 Δmet13(41,1760)::loxP-KanMX4-loxP ΔΔrdn::pNOY455 +* pPK501 (1-4) | This work |
| CEN.BM52-1C | *MATa ura3-52 trp1-289 leu2-3,112 his3Δ1 MAL2-8C SUC2 nep1$^{D90G}$* | This work |
| CEN.PK935-2B | *MATa URA3 TRP1 LEU2 HIS3 MAL2-8$^C$ SUC2 nep1::(-100,3)loxP-kanMX4-loxP-TDH3p-tc-3xHA* | This work |
| CEN.BM113-4A | *MATa ura3-52 trp1-289 leu2-3,112 his3-Δ1 MAL2-8$^C$ SUC2 nep1$^{D90G}$::(-100,3)loxP-kanMX4-loxP-TDH3p-tc-3xHA* | This work |
| CEN.PK1016-4C | *MATα ura3-52 trp1-289 leu2-3,112 his3-Δ1 MAL2-8$^C$ SUC2 nep1-1$^{ts}$* | This work |
| CEN.PK1016-7A | *MATa ura3-52 trp1-289 leu2-3,112 his3-Δ1 MAL2-8$^C$ SUC2 nep1-1$^{ts}$ snr35::loxP-kanMX4::URA3-loxP* | This work |
| CEN.PK1016-9C | *MATa ura3-52 trp1-289 leu2-3,112 his3-Δ1 MAL2-8$^C$ SUC2 Δsnr35::loxP-kanMX4::URA3-loxP* | This work |

## Table S2. Oligonucleotides

| Oligonucleotide | Sequence |
|---|---|
| SNR35-2del5 | GTATATTTACTCTTTTTATTTCAAATTTTAAAAAAAAACCCCAGCTGAAGCTTCGTACGC |
| SNR35-2del3 | TCAATCTTAACTACGTTATTTCTACGTCAAAATAAGAATAGCATAGGCCACTAGTGGATCTG |
| MET13-S1 | ATGAAGATCACAGAAAAATTAGAGCAACATAGACAGACCTCAGCTGAAGCTTCGTACGC |
| MET13-S2 | TAGGCTTAGTAGGATGGAATGGATTTGATCATCTGGAGAAGCATAGGCCACTAGTGGATCTG |
| Core-NEP-5 | TAAAAAAAATGGGTAGAGACATTAGTGAAGCAAGACCTGAGAGCTCGTTTTCGACACTGG |
| Core-NEP-3 | TGGAGAATCTAGCAACGTCAAAAGACATTGGTGGGTAATATCCTTACCATTAAGTTGATC |
| D90G-e | AGGTTTATTAAAAAAAATGGGTAGAGACATTAGTGAAGCAAGACCTGGTATTACCCACCAA TGTCTTTTGACGTTGCTAGATTCTCCAATCAACA |
| D90G-f | TGTTGATTGGAGAATCTAGCAACGTCAAAAGACATTGGTGGGTAATACCAGGTCTTGCTTCA CTAATGTCTCTACCCATTTTTTTTTAATAAACCT |
| NEP1-Tc-1 | CACCACCTTTGAGGGCGTCTCTAACTCTGGAATCTTCGACAAGCTTCGTACGAGCGTAATC |
| NEP1-Tc-2 | AAAAATTATTGTTCGATGAATATATACAGAGATGTCTTGAGCATAGGCCACTAGTGGATCTG |
| NEPD90G-1 | TGAAGCAAGACCTGGTATTACCCACCAATG |
| NEPD90G-2 | CATTGGTGGGTAATACCAGGTCTTGCTTCA |
| NEP1-NsiI | TGCCATGCATACTTAAACACTATAAAATATTCCAAGC |
| NEP1-BamHI-1 | CATGGGATCCGATTCCAGAGTTAGAGACGC |
| 18S-Mut3 | GCGGCTTAATTTGACVCAACACGGGGAAAC |
| 18S-Mut4 | GTTTCCCCGTGTTGBGTCAAATTAAGCCGC |

**Supplementary References**


1. Eschrich, D., Buchhaupt, M., Kötter, P. and Entian, K.D. (2002) Nep1p (Emg1p), a novel protein conserved in eukaryotes and archaea, is involved in ribosome biogenesis. *Curr Genet*, **40**, 326-338.

2. Entian, K.-D. and Kötter, P. (2007) In Stansfield, I. and Stark, M. J. R. (eds.), *Meth. in Microbiol.* Academic Press Ltd., San Diego, Vol. 36, pp. 629-666.

3. Güldener, U., Heck, S., Fielder, T., Beinhauer, J. and Hegemann, J.H. (1996) A new efficient gene disruption cassette for repeated use in budding yeast. *Nucleic Acids Res*, **24**, 2519-2524.

4. Buchhaupt, M., Kötter, P. and Entian, K.D. (2007) Mutations in the nucleolar proteins Tma23 and Nop6 suppress the malfunction of the Nep1 protein. *FEMS Yeast Res*, **7**, 771-781.

5. Voth, W.P., Jiang, Y.W. and Stillman, D.J. (2003) New 'marker swap' plasmids for converting selectable markers on budding yeast gene disruptions and plasmids. *Yeast*, **20**, 985-993.

6. Storici, F., Lewis, L.K. and Resnick, M.A. (2001) In vivo site-directed mutagenesis using oligonucleotides. *Nat Biotechnol*, **19**, 773-776.

7. Kötter, P., Weigand, J.E., Meyer, B., Entian, K.D. and Suess, B. (2009) A fast and efficient translational control system for conditional expression of yeast genes. *Nucleic Acids Res*, **37**, e120.

8. Wai, H.H., Vu, L., Oakes, M. and Nomura, M. (2000) Complete deletion of yeast chromosomal rDNA repeats and integration of a new rDNA repeat: use of rDNA deletion strains for functional analysis of rDNA promoter elements in vivo. *Nucleic Acids Res*, **28**, 3524-3534.

9. Zueva, V.S., Mankin, A.S., Bogdanov, A.A. and Baratova, L.A. (1985) Specific fragmentation of tRNA and rRNA at a 7-methylguanine residue in the presence of methylated carrier RNA. *Eur J Biochem*, **146**, 679-687.

10. Huh, W.K., Falvo, J.V., Gerke, L.C., Carroll, A.S., Howson, R.W., Weissman, J.S. and O'Shea, E.K. (2003) Global analysis of protein localization in budding yeast. *Nature*, **425**, 686-691.