Supplementary Table 1

Yeast plasmids used in this study

Plasmid	Description	Reference
pRS315-ZUO1	pRS315 carrying *ZUO1* under control of its native promoter; used as template to generate all Zuo1 variants used in this study unless specified otherwise.	1
pRS316-ZUO1	pRS316 carrying *ZUO1* under control of its native promoter; used to construct *zuo1*$_{Asp262/Thr266/Val273Ala}$ mutant.	1
pRS315-ZUO1$_{1-310}$	pRS315-ZUO1 with codons 311-433 deleted.	This study
pRS315-MET3-ZUO1	*ZUO1* under control of *MET3* promoter in pRS315.	This study
pRS416-TEF1-RPL31A	pRS416 carrying *RPL31A* under the control of *TEF1*promoter; used to generate pRS416-TEF1-RPL31A-HA.	This study
pRS416-TEF1-RPL31A-HA	pRS416 carrying *RPL31A* gene with HA-tag coding sequence at its 3'-end, under control of *TEF1* promoter; used to generate all Bpa *RPL31A* substitution mutations for crosslinking.	This study
pRS316-RPL22A-HA	pRS316 with *RPL22A-HA* tagged gene under the control of its native promoter; used to generate all Bpa *RPL22A* point mutations for crosslinking.	This study
ptRNA-Bpa	A 2 micron plasmid encoding a variant tRNA synthetase and $tRNA_{CUA}$ for Bpa incorporation; plasmid with either a *TRP1* or *LEU2* marker was used.	2
pRDN-hyg1	URA3-based 2 micron plasmid carrying a hygromycin-resistant *rDNA* repeat under control of its native promoter.	3
pNOY373	A derivative of the 2 micron plasmid YEp351 (*LEU2*) carrying a single copy of *rDNA* repeat under control of its native promoter; used for generating H24Δ1, H59Δ5 and ES12Δ10.	3
pDB688	Reporter plasmid carrying a translational fusion of Renilla and firefly luciferase genes under the control of the *PGK* promoter.	4
pDB688-Stop	Test plasmid for readthrough assays; existing CAA codon replaced with TAG stop codon and flanking GAT and ACG codons with CAA in the linker region between luciferase genes in pDB688.	This study
pDB688-QQQ	Control plasmid for readthrough assay; generated by substituting CAA for GAT and ACG codons flanking the existing CAA codon in the linker region between Renilla and firefly luciferase genes in pDB688.	This study
pJD375	Control plasmid for frameshift assays with Renilla and firefly luciferase genes in frame.	5
pYDL-LA	Test plasmid for assaying -1 PRF with firefly luciferase gene in -1 frame to Renilla luciferase gene.	5

References for Supplementary Table 1

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4. Salas-Marco, J. & Bedwell, D.M. Discrimination between defects in elongation fidelity and termination efficiency provides mechanistic insights into translational readthrough. *J Mol Biol* **348**, 801-15 (2005).
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