The complete chemical structure of *Saccharomyces cerevisiae* rRNA: Partial pseudouridylation of U2345 in 25S rRNA by snoRNA snR9

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Supplementary Table 1-1. Oligonucleotides used in this study.

Oligonucleotide	Sequence	DNA/RNA	Note
c5S-69-chimera	AmCmUmCmGmGmUmCmAm(GGCT)CmUmUmAmCmCmAmGmCmUmUm	RNA/DNA	Used for RNase H digestion to produce Fragment H1 or H2, Nm refers to 2'-O-methyl ribonucleotide. Deoxyribonucleotides are indicated in parentheses.
c18S-385-chimera	GmGmCmUmCmCmCmUmCm(TCCG)GmAmAmUmCmGmAmAmCmCmCm	RNA/DNA	Used for RNase H digestion to produce Fragment H3 or H4, Nm refers to 2'-O-methyl ribonucleotide. Deoxyribonucleotides are indicated in parentheses.
c18S-580-chimera	AmGmCmUmGmGmAmAm(TTAC)CmGmCmGmGmCmUmGmCmUmGmGm	RNA/DNA	Used for RNase H digestion to produce Fragment H4 or H5, Nm refers to 2'-O-methyl ribonucleotide. Deoxyribonucleotides are indicated in parentheses.
c18S-734-chimera	AmGmUmCmCmUmGmGm(TTCG)CmCmAmAmGmAmGmCmCmAmCmAm	RNA/DNA	Used for RNase H digestion to produce Fragment H5 or H6. Nm, 2'-O-methyl ribonucleotide. Deoxyribonucleotides are indicated in parentheses.
c18S-1077-chimera	CmUmCmGmUmAmAmGm(GTGC)CmGmAmGmUmGmGmGmUmCmAmUm	RNA/DNA	Used for RNase H digestion to produce Fragment H6 or H7. Nm, 2'-O-methyl ribonucleotide. Deoxyribonucleotides are indicated in parentheses.
c18S-1321-chimera	AmGmGmUmCmUmCmGmUmUmCmGmUm(TATC)GmCmAmAmUmUmAmAmGmCmAm	RNA/DNA	Used for RNase H digestion to produce Fragment H7 or H8. Nm, 2'-O-methyl ribonucleotide. Deoxyribonucleotides are indicated in parentheses.
c25S-461-chimera	AmCmCmCmAmCmAmAmGmGm(AGCA)GmAmGmGmGmCmAmCmAmAm	RNA/DNA	Used for RNase H digestion to produce Fragment H9 or H10. Nm, 2'-O-methyl ribonucleotide. Deoxyribonucleotides are indicated in parentheses.
c25S-747-chimera	UmCmAmGmGmAmUmCmGmGmUmCmGmAm(TTGT)GmCmAmCmCmUm	RNA/DNA	Used for RNase H digestion to produce Fragment H10 or H11. Nm, 2'-O-methyl ribonucleotide. Deoxyribonucleotides are indicated in parentheses.
c25S-1015-chimera	UmCmGmAmCmCmCmCmGmGm(AACC)UmCmUmAmAmUmCmAmUmUmCmGm	RNA/DNA	Nm, 2'-O-methyl ribonucleotide. Nm refers to 2'-O-methyl ribonucleotide. Deoxyribonucleotides are indicated in parentheses.
c25S-1425-chimera	UmGmCmAmCmUmAmGm(AGGC)CmGmUmUmCmGmAmCmCmCmGmAm	RNA/DNA	Used for RNase H digestion to produce Fragment H12 or H13. Nm, 2'-O-methyl ribonucleotide. Deoxyribonucleotides are indicated in parentheses.
c25S-1855-chimera	UmUmCmAmCmCmUmUmGmGm(AGAC)CmUmGmCmUmGmCmGmGmUm	RNA/DNA	Used for RNase H digestion to produce Fragment H13, H14 or H16. Nm, 2'-O-methyl ribonucleotide. Deoxyribonucleotides are indicated in parentheses.
c25S-2029-chimera	GmGmCmAmCmGmCmAmAmGm(TAGT)CmCmGmCmCmUmAmGmCmAm	RNA/DNA	Used for RNase H digestion to produce Fragment H14 or H15, Nm refers to 2'-O-methyl ribonucleotide. Deoxyribonucleotides are indicated in parentheses.
c25S-2286-chimera	GmAmUmGmAmCmGm(AGGC)AmUmUmUmGmGmCmUmAmCmCmUmUmAm	RNA/DNA	Used for RNase H digestion to produce Fragment H15, H16 or H17. Nm, 2'-O-methyl ribonucleotide. Deoxyribonucleotides are indicated in parentheses.
c25S-2581-chimera	UmCmAmAmCmCmCmGmGm(ATCA)GmCmCmCmCmGmAmAmUmGmGm	RNA/DNA	Used for RNase H digestion to produce Fragment H17 or H18. Nm, 2'-O-methyl ribonucleotide. Deoxyribonucleotides are indicated in parentheses.
c25S-2891-chimera	UmUmCmGmGmUm(ATGA)UmAmGmGmAmAmGmAmGmCm	RNA/DNA	Used for RNase H digestion to produce Fragment H18 or H19. Nm, 2'-O-methyl ribonucleotide.Deoxyribonucleotides are indicated in parentheses.
c25S-2963-chimera	CmCmUmGmUmCmUmCmAmCm(GACG)GmUmCmUmAmAmAmCmCmCmAm	RNA/DNA	Used for RNase H digestion to produce Fragment H20, Nm refers to 2'-O-methyl ribonucleotide. Deoxyribonucleotides are indicated in parentheses.
snR9_F_KO	ATCTTAAGTTTCTTCCCATTTTCCTCCTTCTCTTTCTTCTTCTTTCTTTCCTAAAACCTCGTTGTAAAACGACGGCCAC	DNA	Used for PCR-amplification of the cDNA carrying a snoRNA disrupted by *his3*.
snR9_R_KO	CAAGAGTTAAGAAAGCTTAAAAACGCTATGTAACTGGCATGCAGTCTAAATATTTGCAAACACAGGAAACAGCTATG	DNA	Used for PCR-amplification of the cDNA carrying a snoRNA disrupted by *his3*.
snR33_F_KO	TTCGTTATGAAAATTTTCTACTCTCTTTCACATTTTTTTTTTTCATAAGAATTAAAAAAATGTTGTAAAACGACGGCCAG	DNA	Used for PCR-amplification of the cDNA carrying a snoRNA disrupted by *his3*.
snR33_R_KO	AAAAAGTATACAGATAAACAAGCTCAGTAGTAATACATAAAATAAAAAGTTTTGCAAATCCACAGGAAACAGCTATGA	DNA	Used for PCR-amplification of the cDNA carrying a snoRNA disrupted by *his3*.
snR65_F_KO	TTTCCACGTATTTTAAAAAGGGGTAGCCTGCTTCACCAGATGTTCTTGTCAACAAAATTCGTTGTAAAACGACGGCC	DNA	Used for PCR-amplification of the cDNA carrying a snoRNA disrupted by *his3*.
snR65_R_KO	CGATTTGATTAACGTCATTACGGAAATGTAAGGACACAATTACCAAGAGTTACAAAATCGCACAGGAAACAGCTATG	DNA	Used for PCR-amplification of the cDNA carrying a snoRNA disrupted by *his3*.
snR9_F_Ex	GCGTCGACGGGAATATAATACTAAATACTCTGTTATATAGAACTTTCTACGCCTTTTCCTCTATGGCTAT	DNA	Used for PCR-amplification of the snR9 cDNA.
snR9_R1_Ex	CCATATTCAGTAATTGGTCTATTTGTCTCCGTTGTCCACGCTTTCATAGCCATAGAGGAAAAGGC	DNA	Used for PCR-amplification of the snR9 cDNA.
snR9_R2_Ex	GTCAGGATATCAGCTTAATCCTCACCCATGAAGAAGAAGATATGTCAAAAGCCATATTCAGTAATTGGTCT	DNA	Used for PCR-amplification of the snR9 cDNA.
snR9_R3_Ex	GTCTGCAGAGTATGTCTGAAGGACTAATGATAGGTGGGTCAGGATATCAGCTTAATC	DNA	Used for PCR-amplification of the snR9 cDNA.
snR9_A33G_F_Ex	GCGTCGACGGGAATATAATACTAAATACTCTGTTATATAGgACTTTCTACGCCTTTTCCTCTATGGCTAT	DNA	Used for PCR-amplification of the snR9_A33G cDNA.
snR9_G67A_R1_Ex	CCATATTCAGTAATTGGTCTATTTGTCTCCGTTGTCCACGtTTTCATAGCCATAGAGGAAAAGGC	DNA	Used for PCR-amplification of the snR9_G67A cDNA.
snR9_G69A_R1_Ex	CCATATTCAGTAATTGGTCTATTTGTCTCCGTTGTCCAtGCTTTCATAGCCATAGAGGAAAAGGC	DNA	Used for PCR-amplification of the snR9_G69A cDNA.

Supplementary Table 1-2. Composition of culture media used in this study.

Amino acid/ Nucleobase/ Other	U/C-5-D labelling medium (mg/L)	Sythetic medium (mg/L)	YPD (mg/L)
Adenine	-	35	-
Uracil	-	17	-
5-D-Uracil	100	-	-
Arginine	-	17	-
Aspartate	-	84	-
Glutamate	-	84	-
Histidine	-	17	-
Leucine	-	50	-
Lysine	-	25	-
Methionine	-	17	-
Phenylalanine	-	42	-
Serine	-	315	-
Threonine	-	168	-
Tryptophan	-	34	-
Tyrosine	-	25	-
Valine	-	126	-
Alanine	-	35	-
Asparagine	-	35	-
Cysteine	-	35	-
Glutamine	-	35	-
Glycine	-	35	-
Isoleucine	-	35	-
Proline	-	35	-
Yeast extract	-	-	10 (g)
Peptone	-	-	20 (g)
Yeast nitrogen base without amino acids	6.8 (g)	6.7 (g)	-
Glucose	10 (g)	20 (g)	20 (g)

Supplementary Table 1-3. *S. cerevisiae* strains used in this study.

Strain Name	Mating type	Genotype
BY5208	α	*ura3-52 his3-Δ200*
ΔsnR9	α	*ura3-52 his3-Δ200 snR9::CgHIS3*
ΔsnR33	α	*ura3-52 his3-Δ200 snR33::CgHIS3*
ΔsnR65	α	*ura3-52 his3-Δ200 snR65::CgHIS3*
ΔsnR9_2	a	*ura3-52 his3-Δ200 leu2-Δ1 lys2-Δ202 trp1-Δ63 snR9::CgHIS3*

Supplementary Table S2. Position, type, and stoichiometry of modified nucleotides of *S. cerevisiae* rRNAs determined by the SILNAS-MS methodology.

			RNase T1 fragment			RNase A fragment								
RNA	Position of modified nucleotide	Modification	Fragment sequence[a]	Identification method[b]	Position(s) of candidate fragment(s)[c]	Identified fragment[a]	Identification method[b]	Position(s) of candidate fragment(s)[c]	Stoichiometry (%) of the modified nucleotide during *S. crevisiae* logarithmic growth at 30°C	Labeling and digestion methods[d]	Location in the ribosome (interior, 1; exterior, 0)	Position of corresponding nucleotide in *S. pombe* rRNA[e]	PTM for corresponding nucleotide in *S. pombe* rRNA[e]	Stoichiometry (%) of PTM of corresponding residue in *S. pombe* rRNA at 30 °C[e]	Responsible snoRNA or enzyme[f]
5S	1	5′-end triphosphate	**pppGp**	ND	NA	**pppGGUp**	M	1–3	ND	U/C, RNase A	ND	1	pppG	ND	NA
5S	50	pseudouridine	**ΨAGp**	M, 5dU	50–52, 78–80, 83–85	**GΨp**	M	NA	100	G, RNase T1	0	49	no PTM	0	PUS7
5S	121	3′-end OH	**CAAUCU-OH**	M	116–121	**U-OH**	ND	NA	ND	G, RNase T1	ND	119	3′-end OH	ND	NA
5.8S	1	5′-end phosphate	**pAAACUUUCAACAACGp**	M	1–15	**pAAACp**	M	1–4	ND	G, RNase T1	ND	4	5′-end phosphate	ND	NA
5.8S	73	pseudouridine	**AAΨUGp**	A, M, 5dU	71–75	**GAAΨp**	M	70–73, 87–90, 95–98	78	G, RNase T1	0	76	Ψ	76	snR43
5.8S	158	3′-end OH	**UCAUUU-OH**	M	153–158	**U-OH**	ND	NA	ND	G, RNase T1	ND	160	3′-end OH	ND	NA
18S	1	5′-end phosphate	**pUAUCUGp**	M	1–6	**pUp**	ND	NA	ND	G, RNase T1	ND	1	5-end phosphate	ND	NA
18S	28	2′-*O*-methyl A	**UCAUAmUGp**	A, M	24–30	**AmUp**	M	NA	>95	G, RNase T1	1	28	Am	100	snR74
18S	100	2′-*O*-methyl A	**CUCAmUUAAAΨCAGp**	A, M, 5dU	97–109	**AmUp**	M	NA	80	N, RNase T1	1	101	Am	94	snR51
18S	106	pseudouridine	**CUCAmUUAAAΨCAGp**	A, M, 5dU	97–109	**AAAΨp**	M, 5dU	103–106, 299–302, 436–439, 473–476, 525–528, 1344–1347	>95	N, RNase T1	1	107	Ψ	99	snR44
18S	120	pseudouridine	**UUUAΨUUGp**	A, M, 5dU	116–123	**AΨp**	M, 5dU	NA	>95	G, RNase T1	1	121	Ψ	91	snR49
18S	211	pseudouridine	**UAUUUAΨUAGp**	A, M, 5dU	205–214	**AΨp**	M, 5dU	NA	83	G, RNase T1	0	212	no PTM	0	snR49
18S	302	pseudouridine	**UUCAUUCAAAΨUUCUGp**	A, M, 5dU	292–307	**AAAΨp**	M, 5dU	103–106, 299–302, 436–439, 473–476, 525–528, 1344–1347	86	G, RNase T1	1	305	Ψ	42	snR49
18S	414	2′-*O*-methyl C	**CUACCACAUCmCAAGp**	A, M	405–418	**CmCp**	M	NA	>95	G, RNase T1	0	417	Cm	99	U14
18S	420	2′-*O*-methyl A	**AmAGp**	M	197–199, 420–422, 923–925, 992–994, 1151–1153, 1410–1412, 1693–1695, 1790–1792	**AAGGAmAGGCp**	M	416–424	>95	U/C, RNase A	0	423	Am	98	snR52
18S	436	2′-*O*-methyl A	**CAmAAUUACCCAAUCCUAAUUCAGp**	M	435–457	**AmAAUp**	M	103–106, 299–302, 436–439, 473–476, 525–528, 1344–1347	73	G, RNase T1	1	439	Am	89	snR87
18S	466	pseudouridine	**ΨGp**	M, 5dU	NA	**AGΨp**	M, 5dU	19–21, 22–24, 80–82, 108–110, 126–128, 333–335, 464–466, 615–617, 630–632, 884–886, 1013–1015, 1287–1289, 1348–1350, 1583–1585, 1648–1650, 1667–1669	60	U/C, RNase H (Fragment H4)/RNase A	1	469	Ψ	80	snR189
18S	541	2′-*O*-methyl A	**AmACAAUUGp**	A, M	541–548	**GAGGAmACp**	M	537–543	>95	G, RNase T1	0	544	Am	100	snR41
18S	562	2′-*O*-methyl G	**GmUGp**	M	562–564, 902–904, 1048–1050, 1051–1053, 1265–1267, 1268–1270, 1271–1273, 1292–1294, 1352–1354, 1778–1780	**GGmUp**	M	6–8, 142–144, 153–155, 290–292, 325–327, 346–348, 561–563, 576–578, 647–649, 655–657, 823–825, 837–839, 871–873, 1050–1052, 1118–1120, 1267–1269, 1270–1272, 1291–1293, 1351–1353, 1367–1369, 1394–1396, 1512–1514, 1736–1738	67	U/C, RNase H (Fragment H4)/RNase A	1	565	Gm	81	snrR40
18S	578	2′-*O*-methyl U	**UmAAUUCCAG**	M	578–586	**GGUmAAUp**	M	153–158, 576–581, 1512–1517	>95	G, RNase T1	1	581	Um	100	snR77
18S	619	2′-*O*-methyl A	**UUAmAAAAGp**	A, M	617–624	**AmAAAAGCp**	M	619–625	100	G, RNase T1	1	622	no PTM	0	snR47
18S	632	pseudouridine	**ΨUGp**	M, 5dU	8–10, 608–610, 611–613, 632–634, 649–651, 835–837, 1289–1291, 1608–1610	**AGΨp**	M, 5dU	19–21, 22–24, 80–82, 108–110, 126–128, 333–335, 464–466, 615–617, 630–632, 884–886, 1013–1015, 1287–1289, 1348–1350, 1583–1585, 1648–1650, 1667–1669	84	U/C, RNase H (Fragment H5)/RNase A	0	635	Ψ	105	snR161
18S	759	pseudouridine	**AAAAAAUΨAGp**	A, M, 5dU	752–761	**Ψp**	ND	NA	>95	G, RNase T1	0	772	Ψ	99	snR80
18S	766	pseudouridine	**ΨUCAAAGp**	A, M, 5dU	766–772	**GΨp**	M, 5dU	NA	>95	G, RNase T1	0	779	Ψ	99	snR161
18S	796	2′-*O*-methyl A	**AAUAUAUUAmGp**	A, M	788–797	**AmGCp**	M	425–427, 567–569, 570–572, 585–587, 594–596, 796–798, 1357–1359, 1483–1485, 1600–1602	>95	G, RNase T1	0	811	Am	98	snR53
18S	974	2′-*O*-methyl A	**AAmCGp**	M	973–976, 1325–1328	**AAGAAmCp**	M	970–975	94	U/C, RNase A	1	989	Am	97	snR54
18S	999	pseudouridine	**AΨCAGp**	A, M, 5dU	998–1002	**GAΨp**	M, 5dU	10–12, 123–125, 243–245, 246–248, 287–289, 322–324, 377–379, 480–482, 660–662, 858–860, 997–999, 1255–1257, 1299–1301, 1318–1320, 1405–1407, 1435–1437, 1610–1612, 1654–1656	84	G, RNase T1	1	1014	Ψ	105	snR31
18S	1007	2′-*O*-methyl C	**AUACCmGp**	A, M	1003–1008	**CmGUp**	M	114–116, 627–629, 668–670, 852–854, 1007–1009, 1010–1012, 1280–1282, 1530–1532, 1533–1535, 1641–1643, 1759–1761, 1773–1775	>95	G, RNase T1	1	1022	Cm	99	snR79
18S	1126	2′-*O*-methyl G	**CAAGmGp**	A, M	554–557, 1123–1126, 1591–1594	**AAGmGCp**	M	1124–1128	89	U/C, RNase A	1	1142	Gm	108	snR41
18S	1181	pseudouridine	**CΨUAAUUΨGp**	M, 5dU	1180–1188	**Ψp**	ND	NA	>95	N, RNase T1	1	1198	Ψ	99	snR85
18S	1187	pseudouridine	**CΨUAAUUΨGp**	M, 5dU	1180–1188	**Ψp**	ND	NA	>95	N, RNase T1	1	1204	Ψ	97	snR36
18S	1191	1-methyl-3-(3-amino-3-carboxypropyl)pseudouridine	**ACm^{1}acp^{3}ΨCAACACGp**	M	1189–1198	**m1acp3Ψp**	ND	NA	100	G, RNase T1	1	1208	m1acp3Ψ	100	snR35, Nep1and Tsr3
18S	1269	2′-*O*-methyl U	**UmGp**	M	NA	**GGUmGGmUp**	M	1267–1272	>95	N, RNase H (Fragment H7)/RNase A	1	1286	Um	100	snR55

18S	1271	2′-*O*-methyl G	GmUGp	M	562–564, 902–904, 1048–1050, 1051–1053, 1265–1267, 1268–1270, 1271–1273, 1292–1294, 1352–1354, 1778–1780	GGUmGGmUp	M	1267–1272	>95	N, RNase H (Fragment H7)/RNase A	1	1288	Gm	100	snR40
18S	1280	N^4-acetylcytidine	Cac^{4}CGp	M	572–574, 653–655, 1033–1035, 1279–1281, 1403–1405, 1456–1458, 1500–1502	ac^{4}Cp	ND	NA	84	G, RNase H (Fragment H7)/RNase T1	1	1297	ac^{4}C	79	Kre33
18S	1290	pseudouridine	UΨGp	M, 5dU	8–10, 608–610, 611–613, 632–634, 649–651, 835–837, 1289–1291, 1608–1610	Ψp	ND	NA	93	G, RNase H (Fragment H7)/RNase T1	0	1307	Ψ	91	snR83
18S	1415	pseudouridine	UUΨGp	A, M, 5dU	820–823, 1413–1416	Ψp	ND	NA	79	G, RNase T1	0	1435	Ψ	62	snR83
18S	1428	2′-*O*-methyl G	CAAUAACAGmGp	M	1420–1428	AGmGUp	M	1211–1214, 1427–1430, 1776–1779	>95	G, RNase T1	1	1448	Gm	99	snR56
18S	1572	2′-*O*-methyl G	CUCUUCAACGmAGp	A, M	1563–1572	GmAGm^{7}GAAUp	M	1572–1578	100	G, RNase T1	1	1613	Gm	100	snR57
18S	1575	7-methylguanosine	m^{7}GAAUUCCUAGp	M	1576–1584	GmAGm^{7}GAAUp	M	1572–1578	>95	G, RNase T1	1	1616	m^{7}G	100	Bud23-Trm112 complex
18S	1639	2′-*O*-methyl C	CmCCGp	M	644–647, 1639–1642	GCmCp	M	16–18, 307–309, 564–566, 948–950, 1032–1034, 1438–1440, 1622–1624, 1638–1640	79	G, RNase T1	1	1680	Cm	97	snR70
18S	1773	N^4-acetylcytidine	UUUCac^{4}CGp	M	1769–1774	ac^{4}Cp	ND	NA	>95	G, RNase T1	1	1815	ac^{4}C	97	Kre33
18S	1781	N^6,N^6-dimethyladenosine	m$^6_2$Am$^6_2$ACCUGp	M	1781–1786	Gm$^6_2$Am$^6_2$ACp	M	634–637, 732–735, 1324–1327, 1780–1783	90	G, RNase T1	1	1823	m$^6_2$A	94	Dim1
18S	1782	N^6,N^6-dimethyladenosine	m$^6_2$Am$^6_2$ACCUGp	M	1781–1786	Gm$^6_2$Am$^6_2$ACp	M	634–637, 732–735, 1324–1327, 1780–1783	90	G, RNase T1	1	1824	m$^6_2$A	94	Dim1
18S	1800	3′-end OH	AUCAUUA-OH	M	1794–1800	A-OH	ND	NA	ND	G, RNase T1	ND	1842	3′-end OH	ND	NA
25S	1	5′-end phosphate	pUUGp	M	1–3	pUp	ND	NA	ND	G, RNase T1	ND	3	5′-end phosphate	ND	NA
25S	643	1-methyladenosine	m^{1}AAACAmCmGp	M	643–649	Gm^{1}AAACp	M	642–646, 2353–2357	>95	U/C, RNase H (Fragment H10)/RNase A	1	670	m^{1}A	96	Rrp8
25S	647	2′-*O*-methyl A	m^{1}AAACAmCmGp	M	643–649	AmCmGGACp	M	647–652, 2111–2116	>95	N, RNase T1	1	674	Am	71	U18
25S	648	2′-*O*-methyl C	m^{1}AAACAmCmGp	M	643–649	AmCmGGACp	M	647–652, 2111–2116	94	U/C, RNase H (Fragment H10)/RNase A	1	675	no PTM	0	U18
25S	661	2′-*O*-methyl C	UCmUAACGp	A, M	660–666	CmUp	M	NA	74	G, RNase T1	1	688	Cm	74	snR58
25S	774	pseudouridine	AΨUUGp	A, M, 5dU	773–777, 3389–3393	GGAΨp	M, 5dU	767–770, 771–774, 1304–1307, 1615–1618, 1621–1624, 2313–2316, 2915–2918, 3042–3045, 3099–3102, 3174–3177	100	G, RNase T1	1	808	no PTM	0	snR80
25S	803	2′-*O*-methyl G	ACCCGmAAmAGp	M	799–807	GmAAmAGAUp	M	803–809	>95	G, RNase T1	1	837	Gm	101	snR39b
25S	805	2′-*O*-methyl A	ACCCGmAAmAGp	M	799–807	GmAAmAGAUp	M	803–809	>95	N, RNase A	1	839	Am	100	snR39 or snR59
25S	815	2′-*O*-methyl A	AAmCUAUGp	M	814–820	GAAmCp	M	31–34, 345–348, 712–715, 813–816 922–925, 1081–1084, 1150–1153, 1155–1158, 1287–1290, 1415–1418, 1861–1864, 3013–3016, 3110–3113, 3206–3209	89	G, RNase T1	1	849	Am	94	snR60
25S	865	2′-*O*-methyl G	UAGmCGp	A, M	863–867	AGmCp	M	287–289, 342–344, 496–498, 535–537, 557–559, 576–578, 789–791, 864–866, 934–936, 965–967, 1223–1225, 1292–1294, 1447–1449, 1504–1506, 1697–1699, 1755–1757, 1845–1847, 1865–1867, 1976–1978, 2147–2149, 2273–2275, 2350–2352, 2361–2363, 2388–2390, 2552–2554, 2845–2847, 3213–3215, 3221–3223, 3345–3347	78	G, RNase H (Fragment H11)/RNase T1	1	899	no PTM	0	snR50
25S	874	2′-*O*-methyl A	AmCGp	M	202–204, 596–598, 874–876, 1229–1231, 1361–1363, 1388–1390, 1513–1515, 1654–1656, 1785–1787, 1972–1974, 2047–2049, 2176–2178, 2301–2303	GAmCp	M	3–5, 595–597, 748–750, 873–875, 1027–1029, 1033–1035, 1360–1362, 1387–1389, 1784–1786, 2128–2130, 2175–2177, 2259–2261, 2300–2302, 2416–2418, 2427–2429, 2848–2850, 3307–3309	75	G, RNase H (Fragment H11)/RNase T1	1	908	Am	68	snR72
25S	896	2′-*O*-methyl U	AAUUmUGp	A, M	893–898, 2777–2782	UmUp	M	NA	94	G, RNase T1	1	930	Um	72	snR40
25S	906	2′-*O*-methyl G	GmGp	M	NA	AGGmGGCp	M	904–909	100	U/C, RNase A	1	940	Gm	95	snR60
25S	958	pseudouridine	UUUCCCΨCAGp	A, M, 5dU	952–961	Ψp	ND	NA	87	G, RNase T1	1	992	Ψ	100	snR8
25S	964	pseudouridine	AΨAGp	A, M, 5dU	340–343, 963–966, 1877–1880	AGGAΨp	M, 5dU	514–518, 960–964	91	G, RNase H (Fragment H11)/RNase T1	1	998	Ψ	95	snR43
25S	984	pseudouridine	UΨUUAΨGp	A, M, 5dU	983–989	Ψp	ND	NA	100	N, RNase T1	1	1018	no PTM	0	snR8
25S	988	pseudouridine	UΨUUAΨGp	A, M, 5dU	983–989	AΨp	M, 5dU	NA	100	N, RNase T1	1	1022	no PTM	0	snR49
25S	1002	pseudouridine	AAΨGp	A, M, 5dU	282–285, 530–533, 1000–1003, 1096–1099, 1284–1287, 1645–1648, 2205–2208, 2310–2313, 2992–2995	GAAΨp	M, 5dU	824–827, 892–895, 999–1002, 1095–1098, 1283–1286, 1644–1647, 2204–2207, 2309–2312, 2730–2733, 2991–2994, 3180–3183, 3264–3267	85	G, RNase H (Fragment H11)/RNase T1	1	1036	Ψ	106	snR5
25S	1040	pseudouridine	ACCUAUΨCUCAAACUUΨAAAΨAUGp	M, 5dU	1034–1057	Ψp	ND	NA	>95	N, RNase T1	1	1074	Ψ	100	snR33
25S	1050	pseudouridine	ACCUAUΨCUCAAACUUΨAAAΨAUGp	M, 5dU	1034–1057	Ψp	ND	NA	>95	N, RNase T1	1	1083	Ψ	92	snR81
25S	1054	pseudouridine	ACCUAUΨCUCAAACUUΨAAAΨAUGp	M, 5dU	1034–1057	AAAΨp	M, 5dU	9–12, 112–115, 304–307, 321–324, 519–522, 880–883, 1051–1054, 1450–1453, 1458–1461, 2277–2280, 3292–3295	>95	N, RNase T1	1	1088	no PTM	0	snR44
25S	1108	pseudouridine	CUUΨUAGp	A, M, 5dU	1105–1111	Ψp	ND	NA	70	G, RNase T1	1	1141	no PTM	0	snR82
25S	1122	pseudouridine	CCAUUUΨUGp	A, M, 5dU	1116–1124	Ψp	ND	NA	>95	G, RNase T1	1	1155	Ψ	100	snR5
25S	1131	2′-*O*-methyl A	CAmGp	M	286–288, 513–515, 967–969, 1130–1132, 1370–1372, 1430–1432, 1506–1508, 1572–1574, 1844–1846, 1847–1849, 1975–1977, 2381–2383, 2644–2646, 2830–2832, 2897–2899	AmGAACp	M	1131–1135, 2102–2106, 2687–2691, 3137–3141	88	U/C, RNase A	1	1164	Am	93	snR61

25S	1435	2′-*O*-methyl C	**AUCmUUGp**	A, M	1433–1438	**CmUp**	M	NA	>95	G, RNase T1	1	1471	Cm	92	U24
25S	1447	2′-*O*-methyl A	**UAmGmCAAAUAUUCAAAUGp**	M	1446–1462	**AmGmCp**	M	287–289, 342–344, 496–498, 535–537, 557–559, 576–578, 789–791, 864–866, 934–936, 965–967, 1223–1225, 1292–1294, 1447–1449, 1504–1506, 1697–1699, 1755–1757, 1845–1847, 1865–1867, 1976–1978, 2147–2149, 2273–2275, 2350–2352, 2361–2363, 2388–2390, 2552–2554, 2845–2847, 3213–3215, 3221–3223, 3345–3347	>95	N, RNase H (Fragment H13)/RNase T1	1	1483	Am	100	U24
25S	1448	2′-*O*-methyl G	**UAmGmCAAAUAUUCAAAUGp**	M	1446–1462	**AmGmCp**	M	287–289, 342–344, 496–498, 535–537, 557–559, 576–578, 789–791, 864–866, 934–936, 965–967, 1223–1225, 1292–1294, 1447–1449, 1504–1506, 1697–1699, 1755–1757, 1845–1847, 1865–1867, 1976–1978, 2147–2149, 2273–2275, 2350–2352, 2361–2363, 2388–2390, 2552–2554, 2845–2847, 3213–3215, 3221–3223, 3345–3347	>95	G, RNase T1	1	1484	Gm	99	U24
25S	1886	2′-*O*-methyl U	**AAUAAUmGp**	A, M	1881–1887	**AAUmGUp**	M	1884–1888, 2180–2184, 3271–3275	>95	G, RNase T1	1	1943	Um	97	snR62
25S	2127	pseudouridine	**AAUCΨGp**	A, M, 5dU	2123–2128	**Ψp**	ND	NA	91	G, RNase H (Fragment H15)/RNase T1	1	2217	Ψ	98	snR3
25S	2131	pseudouridine	**ACΨGp**	A, M, 5dU	583–586, 590–593, 1475–1478, 1993–1996, 2129–2132	**Ψp**	ND	NA	100	G, RNase H (Fragment H15)/RNase T1	1	2221	Ψ	103	snR3
25S	2140	1-methyladenosine	**UCUAAUUm^{1}AAAACAUAGp**	A, M	2133–2148	**m^{1}AAAACp**	M	688–692, 2140–2144	>95	G, RNase T1	1	2230	m^{1}A	100	Bmt2
25S	2189	pseudouridine	**AUUΨCUGp**	M, 5dU	2186–2192	**Ψp**	ND	NA	>95	G, RNase T1	1	2279	Ψ	97	snR32
25S	2195	2′-*O*-methyl C	**CCCmAGp**	A, M	2193–2197	**CmAGUp**	M	501–504, 980–983, 1383–1386, 2195–2198, 2477–2480, 2683–2686, 2724–2727	91	G, RNase T1	1	2285	no PTM	0	snR76
25S	2218	2′-*O*-methyl A	**AAmGp**	M	97–99, 263–265, 420–422, 834–836 949–951, 970–972, 1062–1064, 1100–1102, 1242–1244, 1391–1393, 1472–1474, 1479–1481, 1543–1545, 1747–1749, 1807–1809, 1898–1900	**GAAmGAAAUp**	M	2216–2223	93	U/C, RNase A	0	2308	Am	66	snR47
25S	2254	2′-*O*-methyl A	**UAAmCΨAΨGp**	A, M, 5dU	2252–2259	**AAmCp**	M	68–70, 86–88, 162–164, 663–665, 1268–1270, 1271–1273, 1501–1503, 1635–1637, 1640–1642, 1694–1696, 1839–1841, 1919–1921, 2090–2092, 2097–2099, 2226–2228, 2253–2255, 2318–2320, 2640–2642, 2817–2819, 3224–3226, 3320–3322	100	N, RNase T1	1	2344	Am	69	snR63
25S	2256	pseudouridine	**UAAmCΨAΨGp**	A, M, 5dU	2252–2259	**Ψp**	ND	NA	>95	N, RNase T1	1	2346	Ψ	100	snR191
25S	2258	pseudouridine	**UAAmCΨAΨGp**	A, M, 5dU	2252–2259	**AΨp**	M, 5dU	NA	>95	N, RNase T1	1	2348	no PTM	0	snR191
25S	2262	pseudouridine	**ACΨCΨCUUAAGp**	M, 5dU	2260–2270	**Ψp**	ND	NA	>95	N, RNase T1	1	2352	Ψ	100	snR3
25S	2264	pseudouridine	**ACΨCΨCUUAAGp**	M, 5dU	2260–2270	**Ψp**	ND	NA	>95	N, RNase T1	1	2354	Ψ	100	snR84
25S	2276	5-methylcytidine	**Cm^{5}CAAmAmUGp**	M	2275–2281	**m^{5}Cp**	ND	NA	100	N, RNase T1	1	2366	m^{5}C	100	Rcm1
25S	2278	2′-*O*-methyl A	**Cm^{5}CAAmAmUGp**	M	2275–2281	**AAmAmUp**	M	9–12, 112–115, 304–307, 321–324, 519–522, 880–883, 1051–1054, 1450–1453, 1458–1461, 2277–2280, 3292–3295	100	N, RNase T1	1	2368	no PTM	0	snR13
25S	2279	2′-*O*-methyl A	**Cm^{5}CAAmAmUGp**	M	2275–2281	**AAmAmUp**	M	9–12, 112–115, 304–307, 321–324, 519–522, 880–883, 1051–1054, 1450–1453, 1458–1461, 2277–2280, 3292–3295	100	N, RNase T1	1	2369	Am	74	snR13
25S	2286	2′-*O*-methyl G	**CCUCGmUCAUCUAAUUAGp**	M	2282–2298	**GmUp**	M	NA	>95	G, RNase T1	1	2376	Gm	99	snR75
25S	2312	pseudouridine	**AAΨGp**	A, M, 5dU	282–285, 530–533, 1000–1003, 1096–1099, 1284–1287, 1645–1648, 2205–2208, 2310–2313, 2992–2995	**GAAΨp**	M, 5dU	824–827, 892–895, 999–1002, 1095–1098, 1283–1286, 1644–1647, 2204–2207, 2309–2312, 2730–2733, 2991–2994, 3180–3183, 3264–3267	100	G, RNase H (Fragment H17)/RNase T1	1	2402	Ψ	98	snR86
25S	2335	2′-*O*-methyl C	**UCmCCΨAUCUACΨmAΨCΨAGp**	M, 5dU	2334–2351	**CmCp**	M	NA	100	N, RNase T1	1	2425	Cm	91	snR64
25S	2338	pseudouridine	**UCmCCΨAUCUACΨmAΨCΨAGp**	M, 5dU	2334–2351	**Ψp**	ND	NA	100	N, RNase T1	1	2428	no PTM	0	snR9
25S	2345	2′-*O*-methylated pseudouridine	**UCmCCΨAUCUACΨmAΨCΨAGp**	M, 5dU	2334–2351	**ΨmAΨp**	M, 5dU	184–186, 554–556, 625–627, 669–671, 817–819, 986–988, 1037–1039, 1327–1329, 1568–1570, 1680–1682, 1701–1703, 1720–1722, 1738–1740, 2256–2258, 2338–2340, 2345–2347, 2497–2499, 2512–2514, 2755–2757, 2884–2886, 3103–3105	in Table 2	N, RNase T1	1	2435	Ψ	62	snR9^D and snR65
25S	2347	pseudouridine	**UCmCCΨAUCUACΨmAΨCΨAGp**	M, 5dU	2334–2351	**UmAΨp**	M, 5dU	184–186, 554–556, 625–627, 669–671, 817–819, 986–988, 1037–1039, 1327–1329, 1568–1570, 1680–1682, 1701–1703, 1720–1722, 1738–1740, 2256–2258, 2338–2340, 2345–2347, 2497–2499, 2512–2514, 2755–2757, 2884–2886, 3103–3105	100	N, RNase T1	1	2437	Ψ	100	snR82
25S	2349	pseudouridine	**UCmCCΨAUCUACΨmAΨCΨAGp**	M, 5dU	2334–2351	**Ψp**	ND	NA	100	N, RNase T1	1	2439	Ψ	100	snR82
25S	2414	pseudouridine	**CΨUmGp**	A, M, 5dU	537–540, 1779–1782, 1986–1989, 1997–2000, 2007–2010, 2078–2081, 2376–2379, 2413–2416, 2823–2826, 3255–3258	**Ψp**	ND	NA	>95	N, RNase H (Fragment H17) RNase T1	1	2504	Ψ	100	snR11
25S	2415	2′-*O*-methyl U	**CΨUmGp**	A, M, 5dU	537–540, 1779–1782, 1986–1989, 1997–2000, 2007–2010, 2078–2081, 2376–2379, 2413–2416, 2823–2826, 3255–3258	**UmGACp**	M	2–5, 872–875, 1032–1035, 1359–1362, 2127–2130, 2174–2177, 2415–2418, 2426–2429	100	U/C, RNase H (Fragment H17)/RNase A	1	2505	Um	69	snR66
25S	2419	2′-*O*-methyl U	**ACUmCUAGp**	A, M	2417–2423	**UmCp**	M	NA	>95	G, RNase T1	1	2509	Um	100	snR78
25S	2617	2′-*O*-methyl G	**GmGp**	M	NA	**GGmGGCp**	M	168–172, 723–727, 2003–2007, 2571–2575, 2616–2620	87	U/C, RNase H (Fragment H18)/RNase A	1	2714	Gm	97	snR67
25S	2632	3-methyluridine	**Um^{3}UAAACGp**	A, M	2631–2637	**m^{3}UAAACp**	M	2632–2636	>95	G, RNase T1	1	2729	m^{3}U	99	Bmt5
25S	2638	2′-*O*-methyl A	**AmUAACGp**	A, M	2638–2643	**GAmUp**	M	339–341, 358–360, 430–432, 752–754, 758–760, 885–887, 1003–1005, 1140–1142, 1347–1349, 1352–1354, 1526–1528, 1563–1565, 1836–1838, 1876–1878, 2093–2095, 2155–2157, 2169–2171, 2185–2187, 2577–2579, 2637–2639, 2712–2714, 2718–2720, 2759–2761, 2861–2863, 2869–2871, 2988–2990, 3067–3069, 3147–3149, 3359–3361, 3388–3390	93	G, RNase T1	1	2735	Am	80	snR68
25S	2722	2′-*O*-methyl U	**AUUUmUCAGp**	A, M	2719–2726	**UmUp**	M	NA	>95	G, RNase T1	1	2819	no PTM	0	snR67
25S	2727	2′-*O*-methyl U	**UmGp**	M	NA	**AGUmGUp**	M	2725–2729	75	U/C, RNase H (Fragment H18)/RNase A	1	2824	Um	72	snR51

25S	2733	pseudouridine	**AAΨACAAACCAUGp**	A, M, 5dU	2731–2743	**GAAΨp**	M, 5dU	824–827, 892–895, 999–1002, 1095–1098, 1283–1286, 1644–1647, 2204–2207, 2309–2312, 2730–2733, 2991–2994, 3180–3183, 3264–3267	85	G, RNase T1	1	2830	no PTM	0	snR189
25S	2789	2′-*O*-methyl G	**CUAGmAGmGp**	M	2786–2792	**AGmAGmGUp**	M	1007–1012, 2788–2793	86	G, RNase H (Fragment H18)/RNase T1	1	2886	Gm	99	snR48
25S	2791	2′-*O*-methyl G	**CUAGmAGmGp**	M	2786–2792	**AGmAGmGUp**	M	1007–1012, 2788–2793	>95	N, RNase H (Fragment H18)/ RNase A	1	2888	Gm	94	snR48
25S	2813	2′-*O*-methyl G	**GmGp**	M	NA	**AGGmGAUp**	M	2811–2816	>95	U/C, RNase A	1	2910	Gm	85	snR38
25S	2824	pseudouridine	**CΨUGp**	A, M, 5dU	537–540, 1779–1782, 1986–1989, 1997–2000, 2007–2010, 2078–2081, 2378–2379, 2413–2416, 2823–2826, 3255–3258	**Ψp**	ND	NA	87	G, RNase H (Fragment H18)/RNase T1	1	2921	Ψ	99	snR34
25S	2841	3-methyluridine	**Um^{3}UCAUAGp**	A, M	2840–2846	**m^{3}UCp**	M	NA	>95	G, RNase T1	0	2938	no PTM	0	Bmt6
25S	2863	pseudouridine	**AΨUCUUm^{5}CGp**	A, M	2862–2869	**GAΨp**	M, 5dU	339–341, 358–360, 430–432, 752–754, 758–760, 885–887, 1003–1005, 1140–1142, 1347–1349, 1352–1354, 1526–1528, 1563–1565, 1836–1838, 1876–1878, 2093–2095, 2155–2157, 2169–2171, 2185–2187, 2577–2579, 2637–2639, 2712–2714, 2718–2720, 2759–2761, 2861–2863, 2869–2871, 2988–2990, 3067–3069, 3147–3149, 3359–3361, 3388–3390	>95	N, RNase T1	1	2960	Ψ	86	snR46
25S	2868	5-methylcytidine	**AΨUCUUm^{5}CGp**	A, M	2862–2869	**m^{5}Cp**	ND	NA	>95	N, RNase T1	1	2965	m^{5}C	100	Nop2
25S	2878	pseudouridine	**CΨCUUCCUAUCAUACCGp**	M, 5dU	2877–2893	**Ψp**	ND	NA	100	G, RNase T1	1	2975	Ψ	91	snR34
25S	2919	2′-*O*-methyl U	**AUUmGmΨ UCACCCACUAAUAGp**	M, 5dU	2917–2935	**UmGmΨp**	M, 5dU	145–147, 180–182, 248–250, 274–276, 409–411, 447–449, 463–465, 561–563, 792–794, 1069–1071, 1781–1783, 1988–1990, 2039–2041, 2042–2044, 2066–2068, 2131–2133, 2332–2334, 2406–2408, 2432–2434, 2595–2597, 2629–2631, 2825–2827, 2919–2921, 3062–3064, 3194–3196, 3300–3302, 3330–3332, 3350–3352, 3353–3355, 3380–3382, 3383–3385	>95	N, RNase H (Fragment H19)/ RNase T1	1	3016	Um	100	snR52
25S	2920	2′-*O*-methyl G	**AUUmGmΨ UCACCCACUAAUAGp**	M, 5dU	2917–2935	**UmGmΨp**	M, 5dU	145–147, 180–182, 248–250, 274–276, 409–411, 447–449, 463–465, 561–563, 792–794, 1069–1071, 1781–1783, 1988–1990, 2039–2041, 2042–2044, 2066–2068, 2131–2133, 2332–2334, 2406–2408, 2432–2434, 2595–2597, 2629–2631, 2825–2827, 2919–2921, 3062–3064, 3194–3196, 3300–3302, 3330–3332, 3350–3352, 3353–3355, 3380–3382, 3383–3385	>95	N, RNase H (Fragment H19)/ RNase T1	1	3017	Gm	100	SPB1
25S	2921	pseudouridine	**AUUmGmΨ UCACCCACUAAUAGp**	M, 5dU	2917–2935	**UmGmΨp**	M, 5dU	145–147, 180–182, 248–250, 274–276, 409–411, 447–449, 463–465, 561–563, 792–794, 1069–1071, 1781–1783, 1988–1990, 2039–2041, 2042–2044, 2066–2068, 2131–2133, 2332–2334, 2406–2408, 2432–2434, 2595–2597, 2629–2631, 2825–2827, 2919–2921, 3062–3064, 3194–3196, 3300–3302, 3330–3332, 3350–3352, 3353–3355, 3380–3382, 3383–3385	>95	N, RNase H (Fragment H19)/ RNase T1	1	3018	Ψ	100	snR10
25S	2942	pseudouridine	**ΨGp**	M, 5dU	NA	**GΨp**	M, 5dU	NA	58	G, RNase H (Fragment H19)/RNase T1	1	3039	Ψ	98	snR37
25S	2944	2′-*O*-methyl A	**AmGp**	M	NA	**GAmGCmUp**	M	2410–2414, 2943–2947	91	G, RNase H (Fragment H19)/RNase T1	1	3041	Am	99	snR71
25S	2946	2′-*O*-methyl C	**CmUGp**	M	29–31, 578–580, 612–614, 791–793 936–938, 1743–1745, 1765–1767, 2529–2531, 2575–2577, 2614–2616, 2946–2948, 3061–3063, 3097–3099, 3108–3110, 3204–3206, 3259–3261	**GAmGCmUp**	M	2410–2414, 2943–2947	>95	G, RNase H (Fragment H19)/RNase T1	1	3043	Cm	82	snR69
25S	2957	2′-*O*-methyl C	**ACmCGp**	A, M	336–339, 749–752, 1399–1402, 2071–2074, 2956–2959	**AGACmCp**	M	1397–1401, 2069–2073, 2954–2958	94	G, RNase H (Fragment H19)/RNase T1	1	3054	Cm	80	snR73
25S	2973	pseudouridine	**UΨAGp**	M, 5dU	1208–1211, 1520–1523, 2972–2975	**Ψp**	ND	NA	>95	G, RNase H (Fragment H19)/RNase T1	1	3070	Ψ	99	snR42
25S	3394	3′-end OH	**U-OH**	ND	NA	**GU-OH**	M	3393–3394	ND	U/C, RNase A	ND	3498	3′-end OH	ND	NA

a. Modified residues are in red.

b. The RNA fragment was identified by Ariadne (A) and/or by manual inspection of MS and MS/MS spectra (M). 5dU, rRNA labeled with 5-D-uracil was used to determine the position of pseudouridines; ND, not detected.

c. Candidates consisting of three or more bases are indicated. The final determined position is underlined. NA, not assigned.

d. Fragment number obtained by RNase H digestion is defined in Supplementary Table 1 and Supplementary Figure S1. G, internal standard labelled by [$^{13}C_{10}$]GTP was used for stoichiometric quantification; U/C, internal standard labeled by [$^{13}C_9$]UTP and [$^{13}C_9$]CTP was used for stoichiometric quantification; N, stoichiometry was determined from the ratio of LC-MS peak areas of the RNA fragments possessing the same RNA backbone (total area of fragments with the modification/that of all fragments) with no isotopic label. ND, not determined.

e. According to Taoka M et al, Nucleic Acids Res. 2015 Oct 15;43(18):e115. NA, not assigned.

f. According to the supplementry table in Sharma S and Lafontaine DL, Trends Biochem Sci. 2015 Oct;40(10):560-75 except for snR9 of U2345 of 25S rRNA. NA, not assigned.

g. Identified in this study.

Supplementary Table S3. Stoichiometries of U and modified nucleoties at positions 2338 and U2345 in *S. cerevisiae* 25S rRNA.

	U2338		U2345			
Plasmid[a]	U %[b]	Ψ %[b]	U %[b]	Um %[b]	Ψ %[b]	Ψm %[b]
pSEC	100.0	0.0	14.8	85.2	0.0	0.0
pSECR9WT	0.0	100.0	12.8	74.5	5.5	7.2
pSECR9A33G	61.0	39.0	24.6	75.4	0.0	0.0
pSECR9G67A	64.2	35.8	20.0	80.0	0.0	0.0
pSECR9G69A	40.9	59.1	13.1	86.9	0.0	0.0

a. Each plasmid was transformed into ΔsnR9_2 that was then cultured in the U/C-5-D-labelling medium containing 0.01% (w/v) 5-D-uracil.
b. The values were calculated from the peak areas obtained by extracted ion monitoring of the most abundunt masses of RNase T1 fragments containing U2338 and U2345 of 25S rRNA. The rRNAs used in this experiment were purified by reversed-phase LC of total