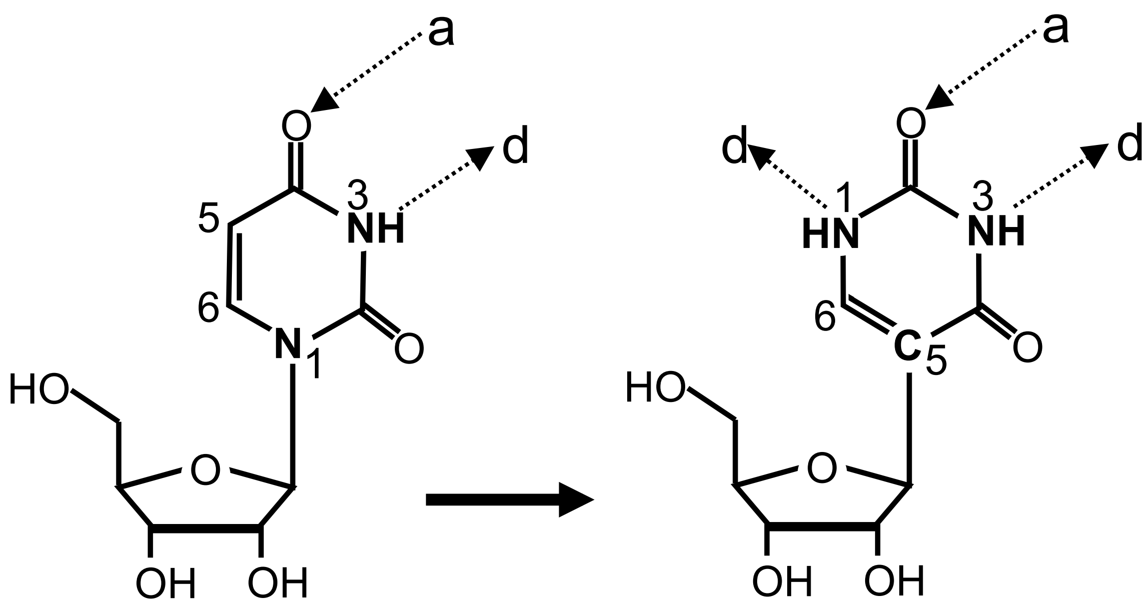
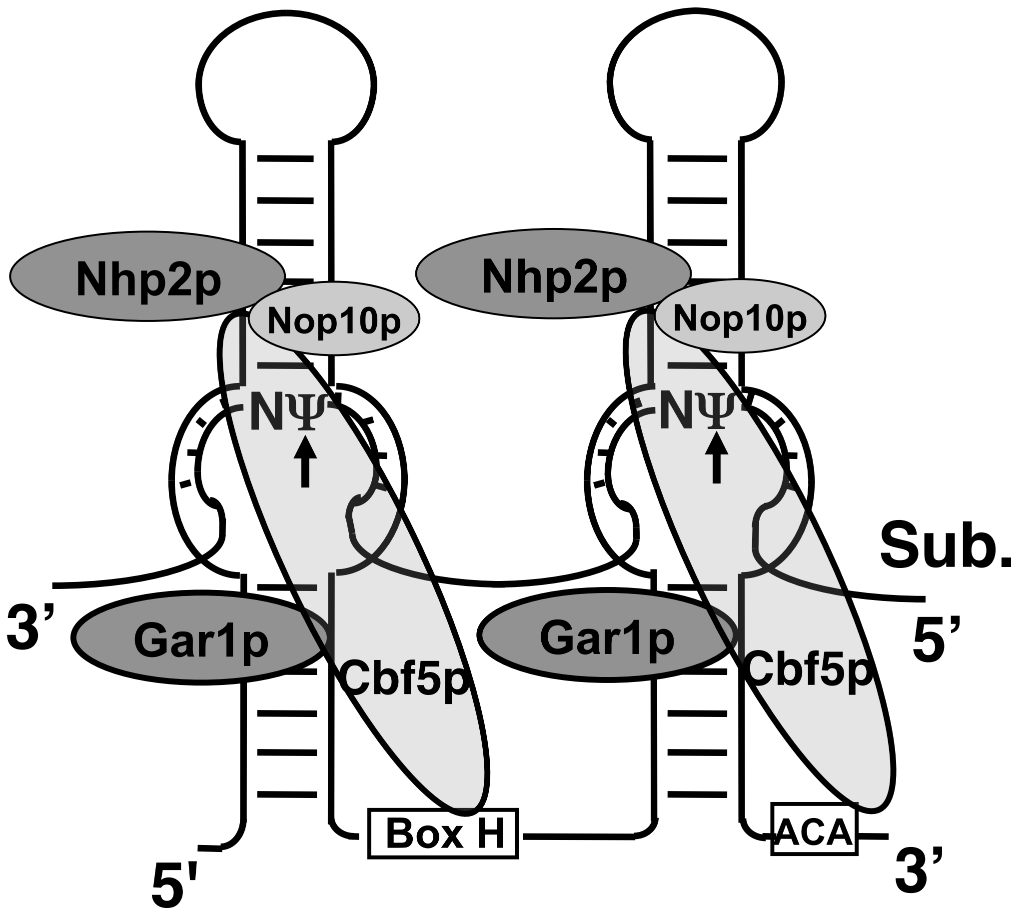


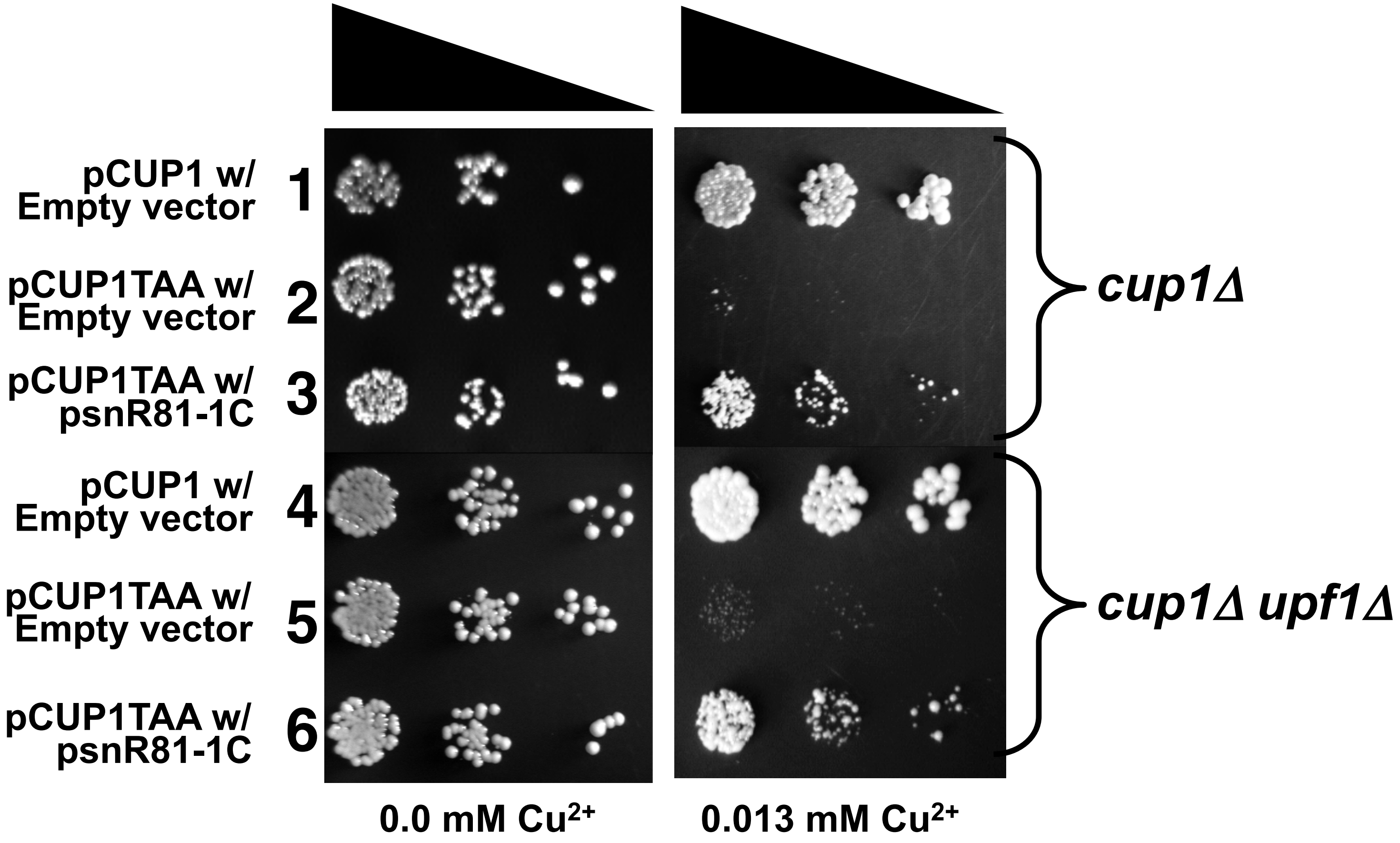
(Uridine) **(Ψ)**

Sup. Fig. 1. Comparison of the structures of uridine and pseudouridine (Ψ). Ψ has one more hydrogen bond donor (d) than uridine, while hydrogen bond acceptors (a) are the same.

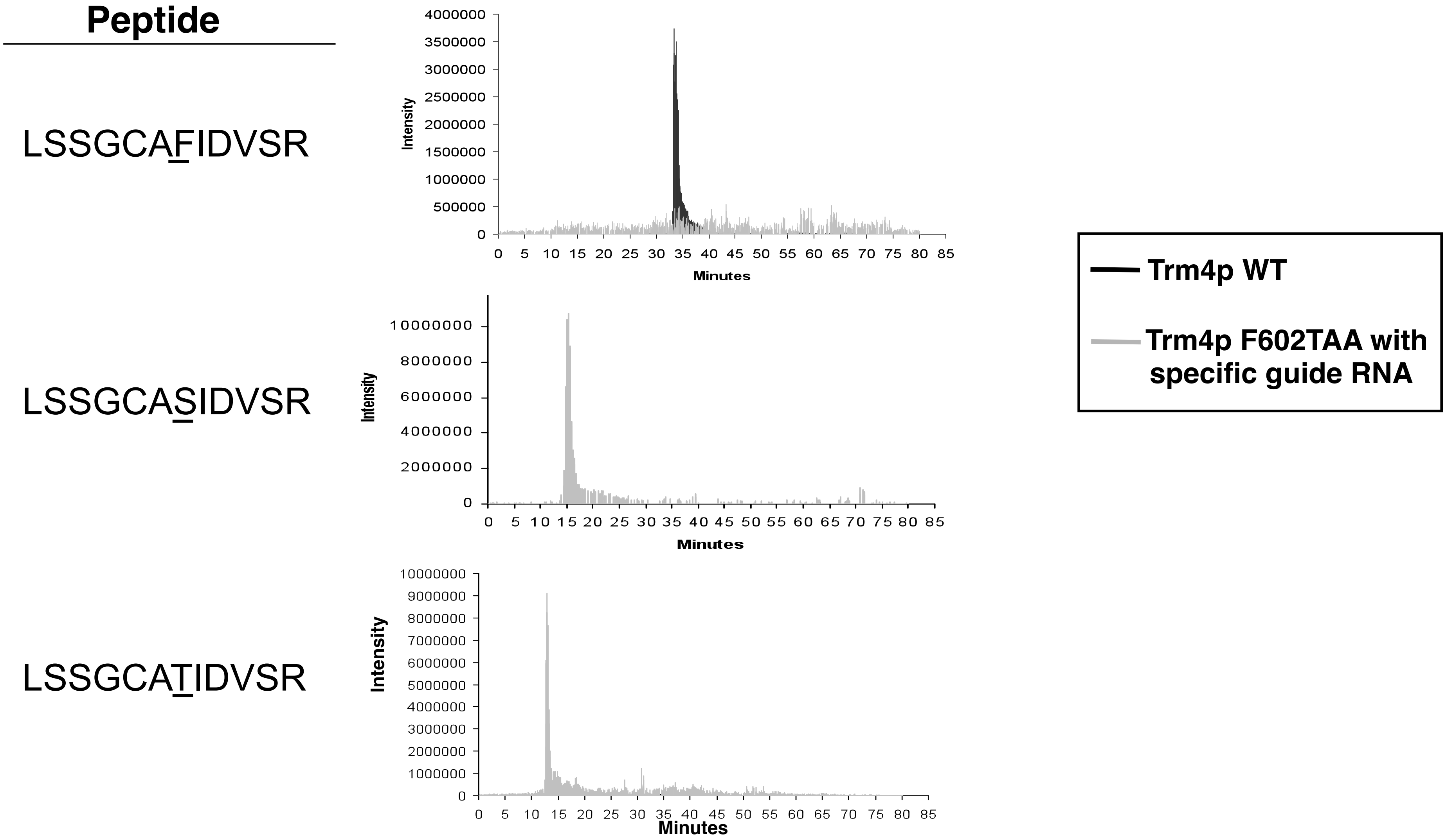
Box H/ACA RNP



Sup. Fig. 2) Schematic of an H/ACA RNP, consisting of a box H/ACA RNA and four core proteins, namely Cbf5p, Gar1p, Nhp2p, and Nop10p. A substrate RNA (Sub.) and the target nucleotide (Ψ) are also shown (adopted and modified from[28]).



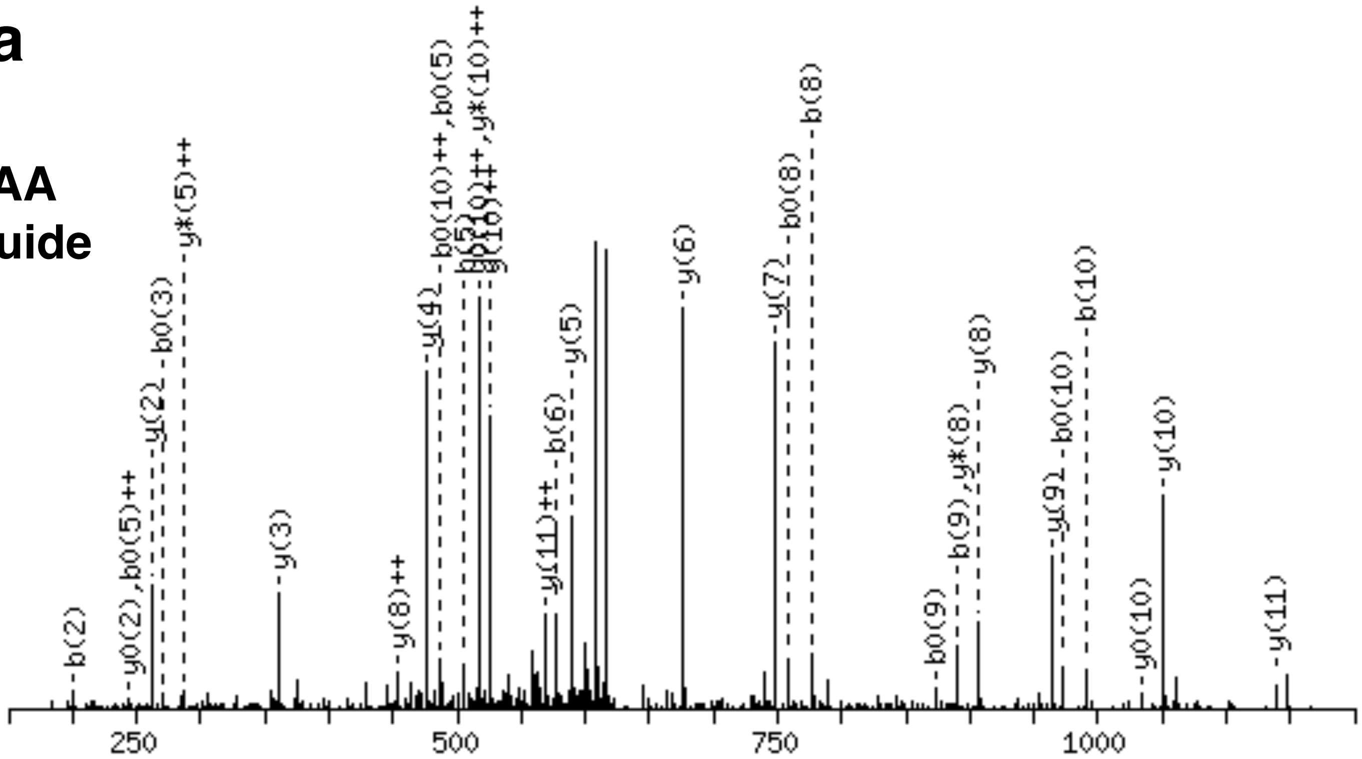
Sup. Fig 3. Deletion of UPF1 results in a minor nonsense suppression phenotype. pCUP1 or pcup1-PTC along with either an empty vector or psnR81-1C were transformed into either a cup1Δ or cup1Δ upf1Δ strain. Cell growth was assessed on solid synthetic medium (-URA -LEU) containing either 0.0 mM or 0.013 mM $CuSO_4$, as indicated.

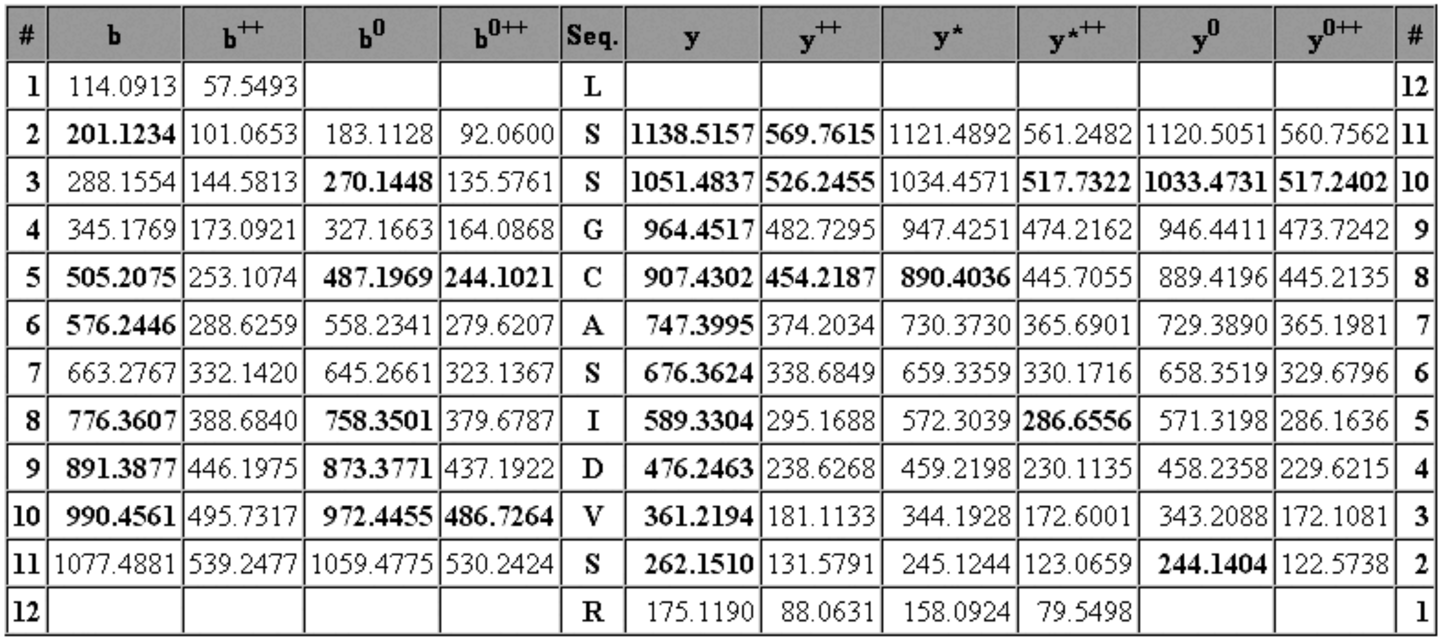


Sup. Fig. 4. ΨAA directs the incorporation of serine and threonine. HPLC traces showing the elution of the novel serine- and threonine-containing peptides in the indicated samples. The underlined amino acid represents the site of the termination codon.

Sup Fig 5a

Trm4p F602TAA
w/ Specific Guide

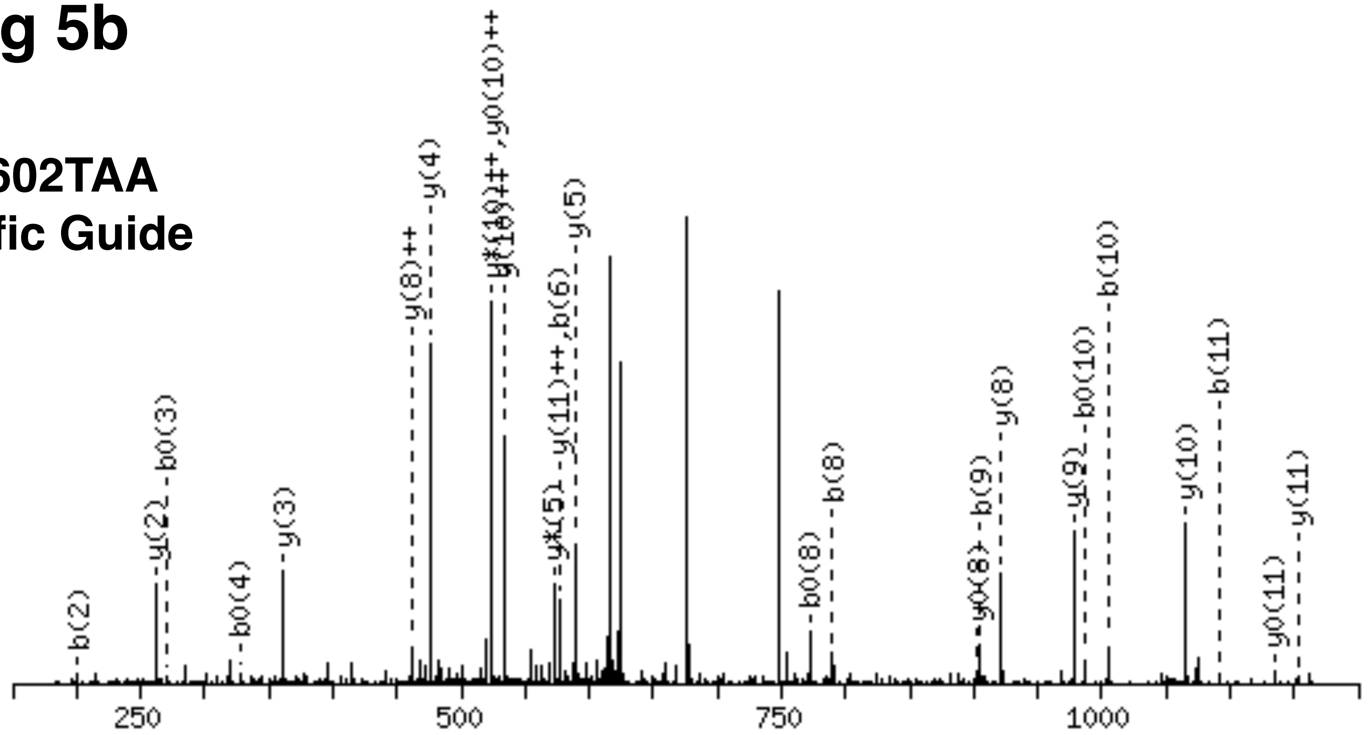




#	b	b^{++}	b^0	b^{0++}	Seq.	y	y^{++}	y^*	y^{*++}	y^0	y^{0++}	#
1	114.0913	57.5493			L							12
2	**201.1234**	101.0653	183.1128	92.0600	S	**1138.5157**	**569.7615**	1121.4892	561.2482	1120.5051	560.7562	11
3	288.1554	144.5813	**270.1448**	135.5761	S	**1051.4837**	**526.2455**	1034.4571	**517.7322**	**1033.4731**	**517.2402**	10
4	345.1769	173.0921	327.1663	164.0868	G	**964.4517**	482.7295	947.4251	474.2162	946.4411	473.7242	9
5	**505.2075**	253.1074	**487.1969**	**244.1021**	C	**907.4302**	**454.2187**	**890.4036**	445.7055	889.4196	445.2135	8
6	**576.2446**	288.6259	558.2341	279.6207	A	**747.3995**	374.2034	730.3730	365.6901	729.3890	365.1981	7
7	663.2767	332.1420	645.2661	323.1367	S	**676.3624**	338.6849	659.3359	330.1716	658.3519	329.6796	6
8	**776.3607**	388.6840	**758.3501**	379.6787	I	**589.3304**	295.1688	572.3039	**286.6556**	571.3198	286.1636	5
9	**891.3877**	446.1975	**873.3771**	437.1922	D	**476.2463**	238.6268	459.2198	230.1135	458.2358	229.6215	4
10	**990.4561**	495.7317	**972.4455**	**486.7264**	V	**361.2194**	181.1133	344.1928	172.6001	343.2088	172.1081	3
11	1077.4881	539.2477	1059.4775	530.2424	S	**262.1510**	131.5791	245.1244	123.0659	**244.1404**	122.5738	2
12					R	175.1190	88.0631	158.0924	79.5498			1

Sup Fig 5b

Trm4p F602TAA
w/ Specific Guide

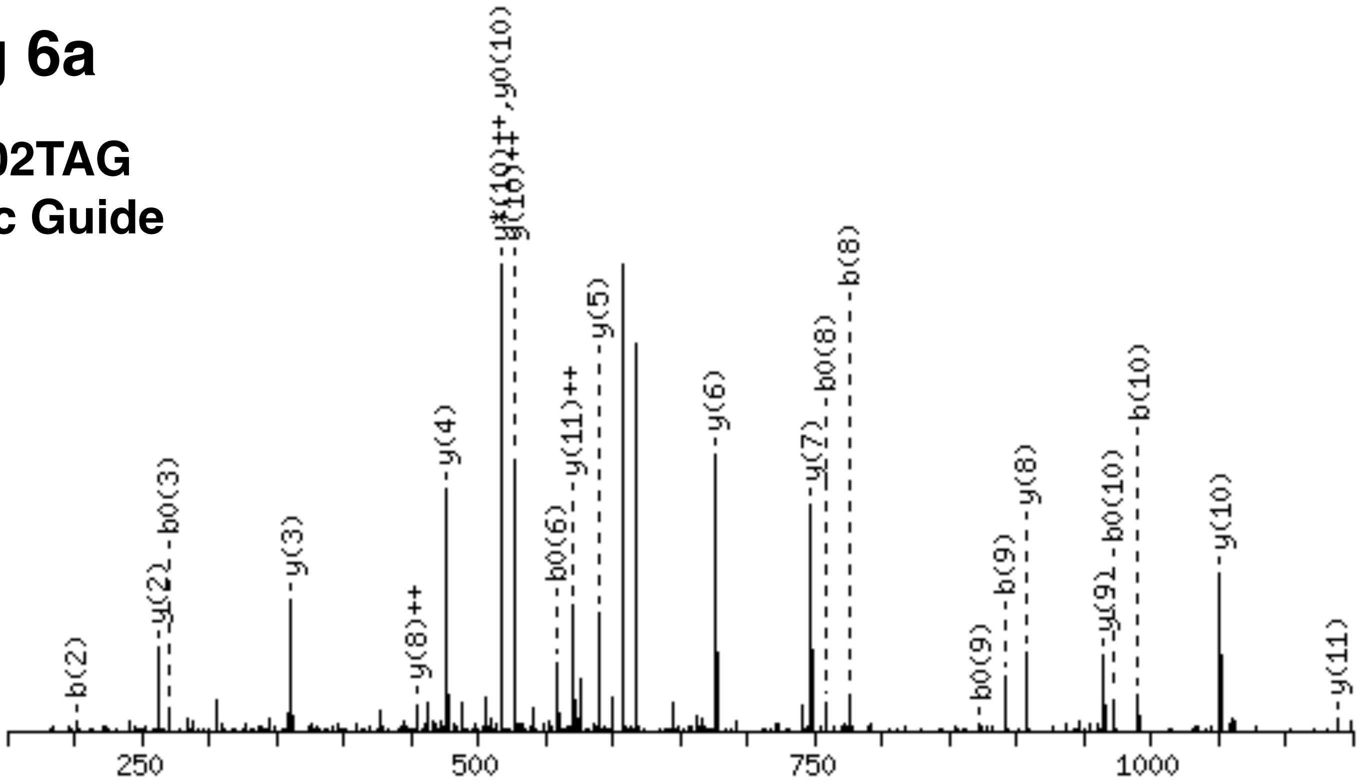


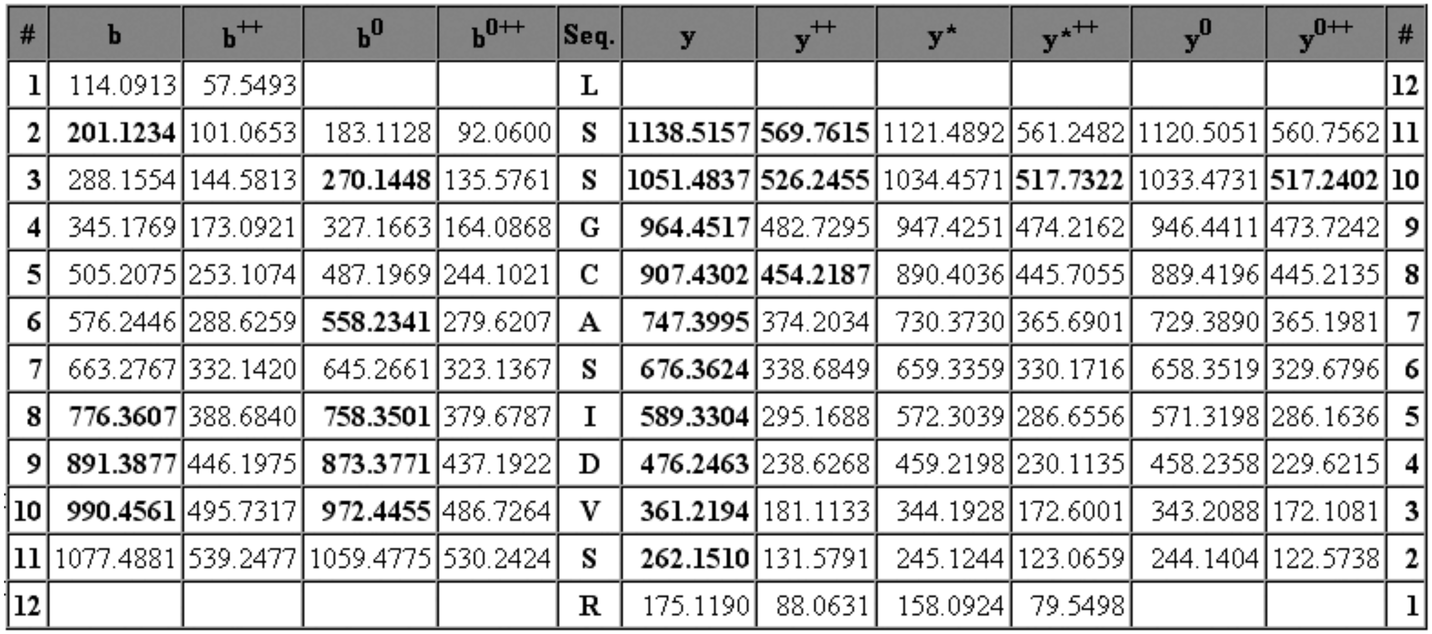
#	b	b^{++}	b^{0}	b^{0++}	Seq.	y	y^{++}	y^{*}	y^{*++}	y^{0}	y^{0++}	#
1	114.0913	57.5493			L							12
2	**201.1234**	101.0653	183.1128	92.0600	S	**1152.5314**	**576.7693**	1135.5048	568.2560	**1134.5208**	567.7640	11
3	288.1554	144.5813	**270.1448**	135.5761	S	**1065.4993**	**533.2533**	1048.4728	**524.7400**	1047.4888	**524.2480**	10
4	345.1769	173.0921	**327.1663**	164.0868	G	**978.4673**	489.7373	961.4408	481.2240	960.4567	480.7320	9
5	505.2075	253.1074	487.1969	244.1021	C	**921.4458**	**461.2266**	904.4193	452.7133	**903.4353**	452.2213	8
6	**576.2446**	288.6259	558.2341	279.6207	A	761.4152	381.2112	744.3886	372.6980	743.4046	372.2060	7
7	677.2923	339.1498	659.2817	330.1445	T	690.3781	345.6927	673.3515	337.1794	672.3675	336.6874	6
8	**790.3764**	395.6918	**772.3658**	386.6865	I	**589.3304**	295.1688	**572.3039**	286.6556	571.3198	286.1636	5
9	**905.4033**	453.2053	887.3927	444.2000	D	**476.2463**	238.6268	459.2198	230.1135	458.2358	229.6215	4
10	**1004.4717**	502.7395	**986.4612**	493.7342	V	**361.2194**	181.1133	344.1928	172.6001	343.2088	172.1081	3
11	**1091.5038**	546.2555	1073.4932	537.2502	S	**262.1510**	131.5791	245.1244	123.0659	244.1404	122.5738	2
12					R	175.1190	88.0631	158.0924	79.5498			1

Sup. Fig 5. Identification of amino acids encoded at pseudouridylated UAA (ΨAA). (a) MS/MS spectrum (top) and mass of all possible ions associated with the serine-containing peptide (bottom). Bold masses represent ions identified. (b) MS/MS spectrum (top) and mass of all possible ions associated with the threonine-containing peptide (bottom). Bold masses represent ions identified.

Sup Fig 6a

Trm4p F602TAG
w/ Specific Guide

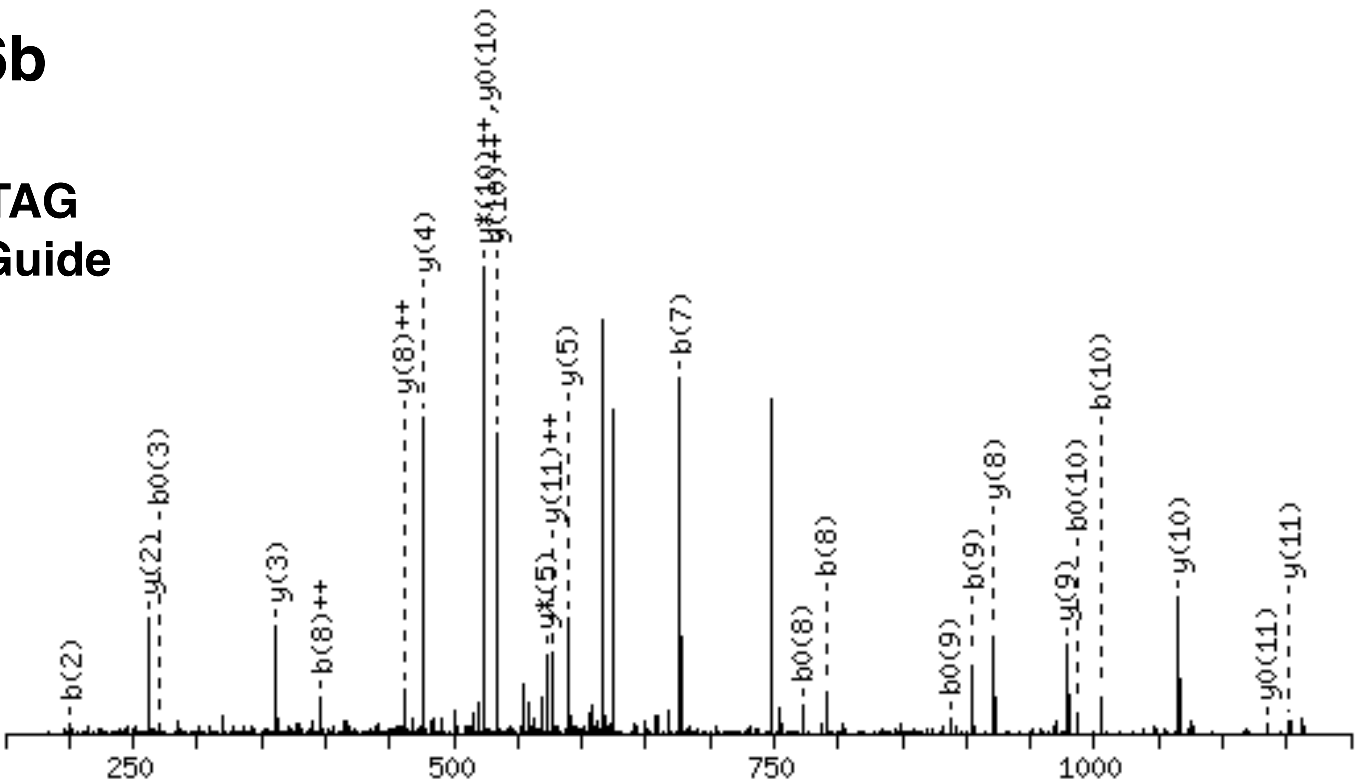


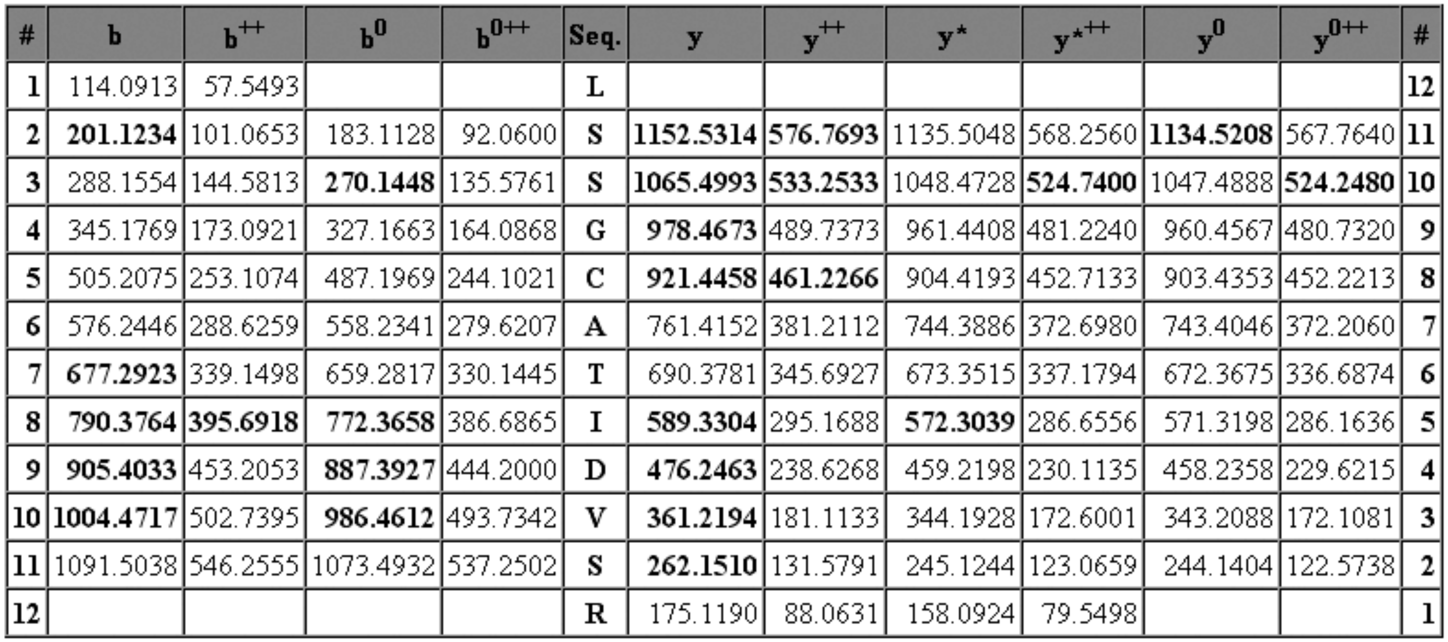


#	b	b^{++}	b^{0}	b^{0++}	Seq.	y	y^{++}	y^{*}	y^{*++}	y^{0}	y^{0++}	#
1	114.0913	57.5493			L							12
2	**201.1234**	101.0653	183.1128	92.0600	S	**1138.5157**	**569.7615**	1121.4892	561.2482	1120.5051	560.7562	11
3	288.1554	144.5813	**270.1448**	135.5761	S	**1051.4837**	**526.2455**	1034.4571	**517.7322**	1033.4731	**517.2402**	10
4	345.1769	173.0921	327.1663	164.0868	G	**964.4517**	482.7295	947.4251	474.2162	946.4411	473.7242	9
5	505.2075	253.1074	487.1969	244.1021	C	**907.4302**	**454.2187**	890.4036	445.7055	889.4196	445.2135	8
6	576.2446	288.6259	**558.2341**	279.6207	A	**747.3995**	374.2034	730.3730	365.6901	729.3890	365.1981	7
7	663.2767	332.1420	645.2661	323.1367	S	**676.3624**	338.6849	659.3359	330.1716	658.3519	329.6796	6
8	**776.3607**	388.6840	**758.3501**	379.6787	I	**589.3304**	295.1688	572.3039	286.6556	571.3198	286.1636	5
9	**891.3877**	446.1975	**873.3771**	437.1922	D	**476.2463**	238.6268	459.2198	230.1135	458.2358	229.6215	4
10	**990.4561**	495.7317	**972.4455**	486.7264	V	**361.2194**	181.1133	344.1928	172.6001	343.2088	172.1081	3
11	1077.4881	539.2477	1059.4775	530.2424	S	**262.1510**	131.5791	245.1244	123.0659	244.1404	122.5738	2
12					R	175.1190	88.0631	158.0924	79.5498			1

Sup Fig 6b

Trm4p F602TAG
w/ Specific Guide



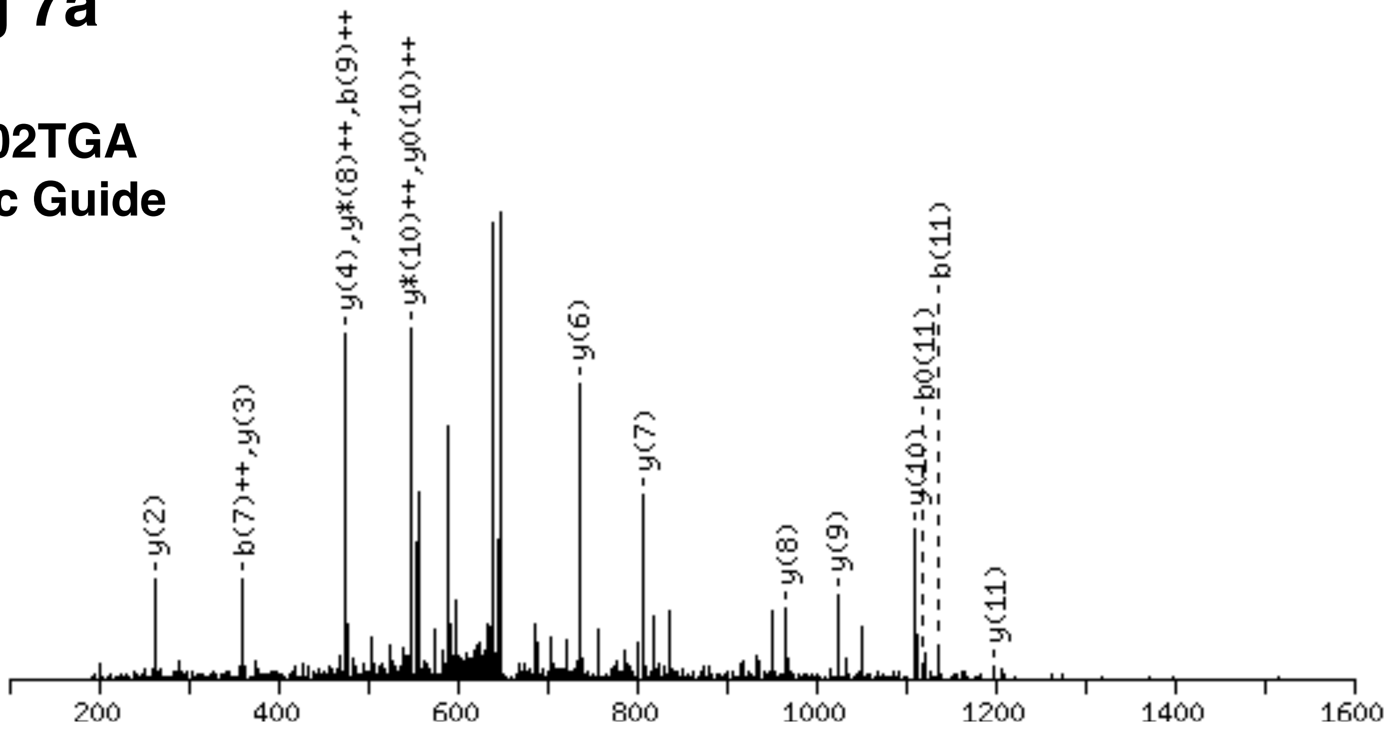


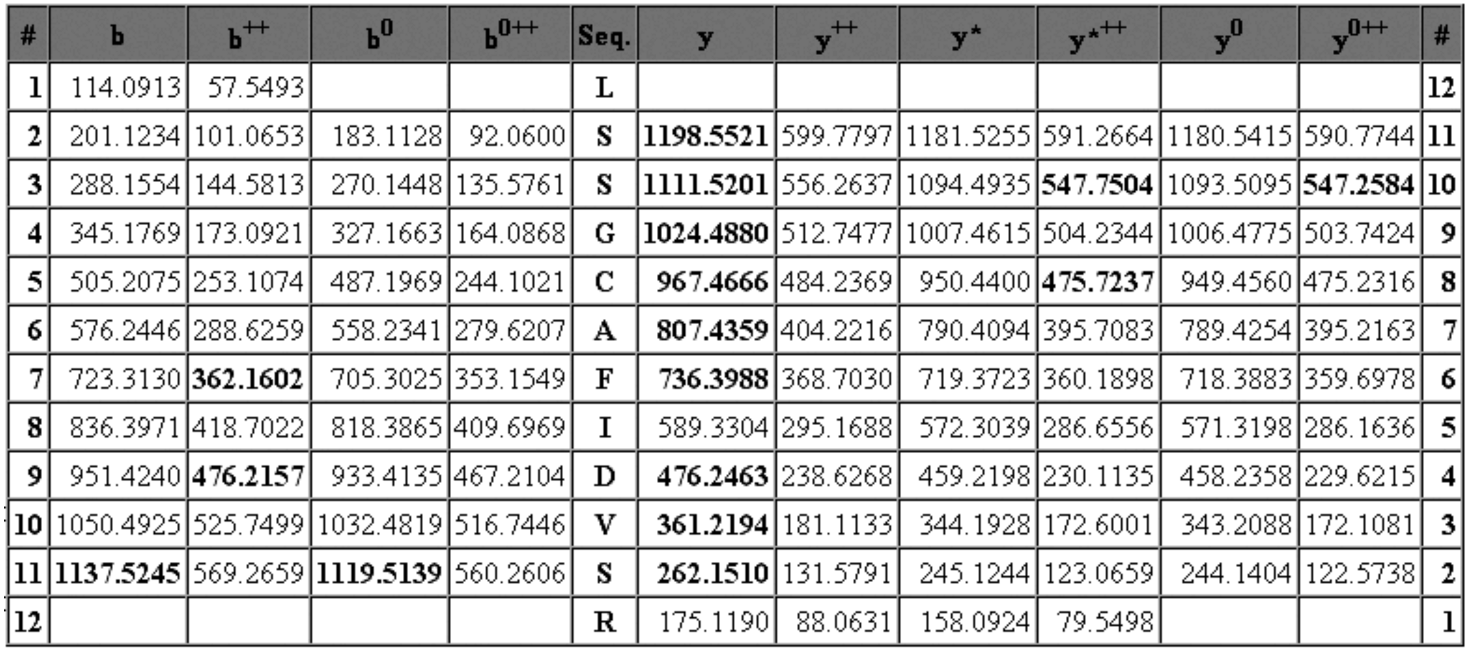
#	b	b^{++}	b^{0}	b^{0++}	Seq.	y	y^{++}	y^{*}	y^{*++}	y^{0}	y^{0++}	#
1	114.0913	57.5493			**L**							**12**
2	**201.1234**	101.0653	183.1128	92.0600	**S**	**1152.5314**	**576.7693**	1135.5048	568.2560	**1134.5208**	567.7640	**11**
3	288.1554	144.5813	**270.1448**	135.5761	**S**	**1065.4993**	**533.2533**	1048.4728	**524.7400**	1047.4888	**524.2480**	**10**
4	345.1769	173.0921	327.1663	164.0868	**G**	**978.4673**	489.7373	961.4408	481.2240	960.4567	480.7320	**9**
5	505.2075	253.1074	487.1969	244.1021	**C**	**921.4458**	**461.2266**	904.4193	452.7133	903.4353	452.2213	**8**
6	576.2446	288.6259	558.2341	279.6207	**A**	761.4152	381.2112	744.3886	372.6980	743.4046	372.2060	**7**
7	**677.2923**	339.1498	659.2817	330.1445	**T**	690.3781	345.6927	673.3515	337.1794	672.3675	336.6874	**6**
8	**790.3764**	**395.6918**	**772.3658**	386.6865	**I**	**589.3304**	295.1688	**572.3039**	286.6556	571.3198	286.1636	**5**
9	**905.4033**	453.2053	**887.3927**	444.2000	**D**	**476.2463**	238.6268	459.2198	230.1135	458.2358	229.6215	**4**
10	**1004.4717**	502.7395	**986.4612**	493.7342	**V**	**361.2194**	181.1133	344.1928	172.6001	343.2088	172.1081	**3**
11	1091.5038	546.2555	1073.4932	537.2502	**S**	**262.1510**	131.5791	245.1244	123.0659	244.1404	122.5738	**2**
12					**R**	175.1190	88.0631	158.0924	79.5498			**1**

Sup. Fig. 6. Identification of amino acids encoded at pseudouridylated UAG (ΨAG). (a) MS/MS spectrum (top) and mass of all possible ions associated with the serine-containing peptide (bottom). Bold masses represent ions identified. (b) MS/MS spectrum (top) and mass of all possible ions associated with the threonine-containing peptide (bottom). Bold masses represent ions identified.

Sup Fig 7a

Trm4p F602TGA
w/ Specific Guide

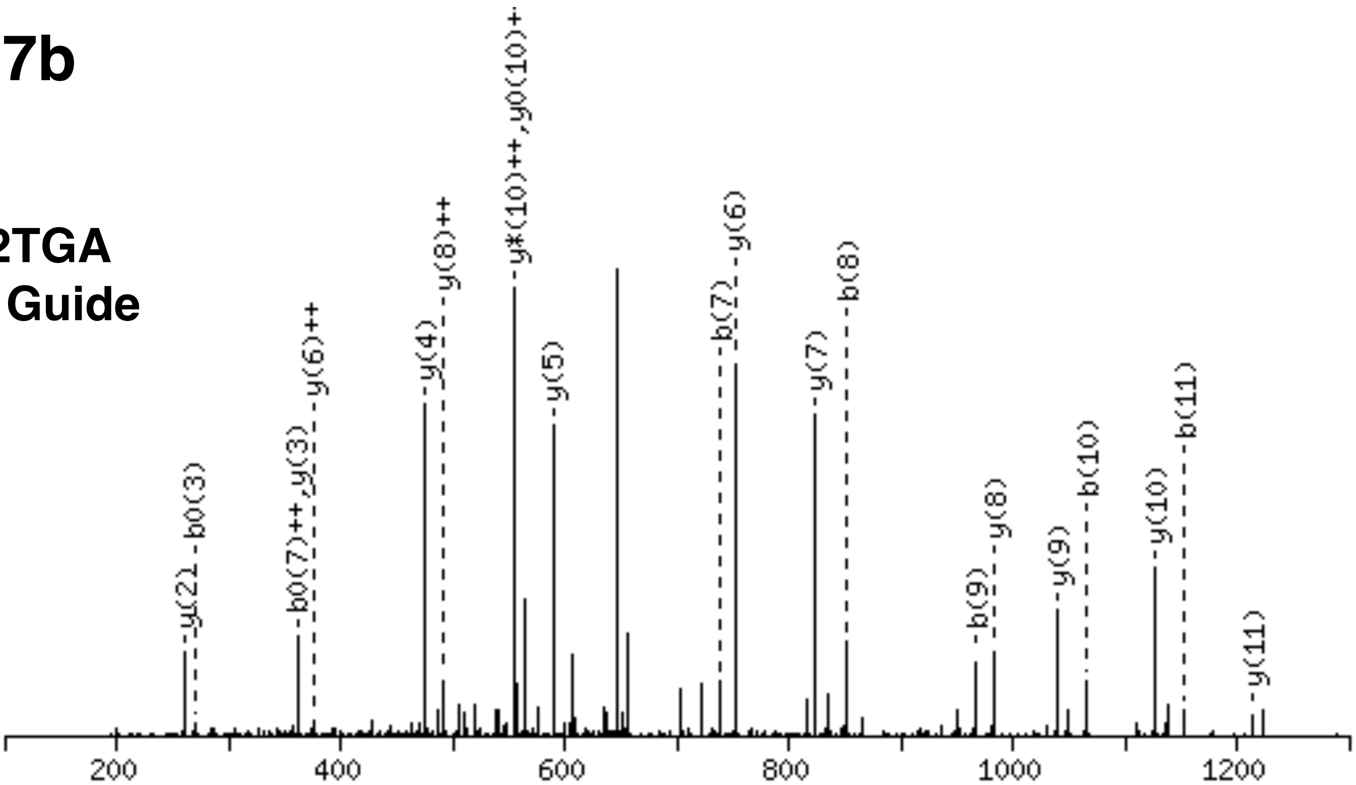


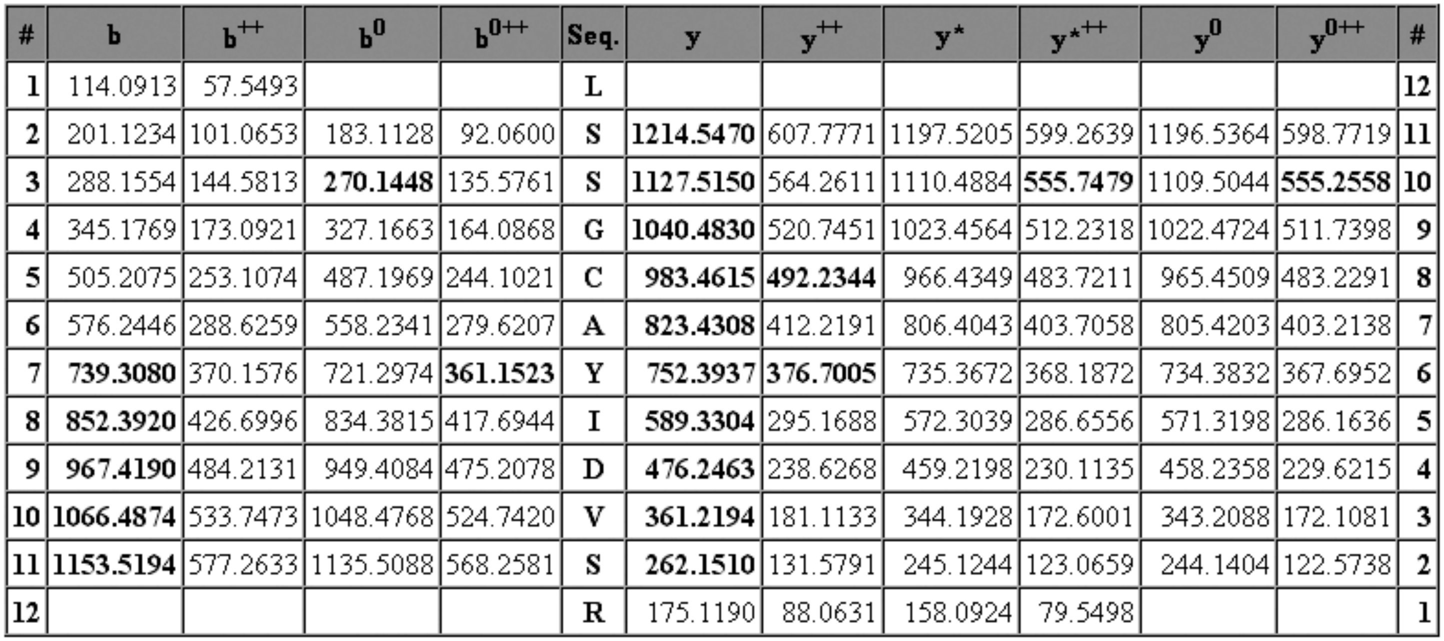


#	b	b^{++}	b^{0}	b^{0++}	Seq.	y	y^{++}	y^{*}	y^{*++}	y^{0}	y^{0++}	#
1	114.0913	57.5493			**L**							**12**
2	201.1234	101.0653	183.1128	92.0600	**S**	**1198.5521**	599.7797	1181.5255	591.2664	1180.5415	590.7744	**11**
3	288.1554	144.5813	270.1448	135.5761	**S**	**1111.5201**	556.2637	1094.4935	**547.7504**	1093.5095	**547.2584**	**10**
4	345.1769	173.0921	327.1663	164.0868	**G**	**1024.4880**	512.7477	1007.4615	504.2344	1006.4775	503.7424	**9**
5	505.2075	253.1074	487.1969	244.1021	**C**	**967.4666**	484.2369	950.4400	**475.7237**	949.4560	475.2316	**8**
6	576.2446	288.6259	558.2341	279.6207	**A**	**807.4359**	404.2216	790.4094	395.7083	789.4254	395.2163	**7**
7	723.3130	**362.1602**	705.3025	353.1549	**F**	**736.3988**	368.7030	719.3723	360.1898	718.3883	359.6978	**6**
8	836.3971	418.7022	818.3865	409.6969	**I**	589.3304	295.1688	572.3039	286.6556	571.3198	286.1636	**5**
9	951.4240	**476.2157**	933.4135	467.2104	**D**	**476.2463**	238.6268	459.2198	230.1135	458.2358	229.6215	**4**
10	1050.4925	525.7499	1032.4819	516.7446	**V**	**361.2194**	181.1133	344.1928	172.6001	343.2088	172.1081	**3**
11	**1137.5245**	569.2659	**1119.5139**	560.2606	**S**	**262.1510**	131.5791	245.1244	123.0659	244.1404	122.5738	**2**
12					**R**	175.1190	88.0631	158.0924	79.5498			**1**

Sup Fig 7b

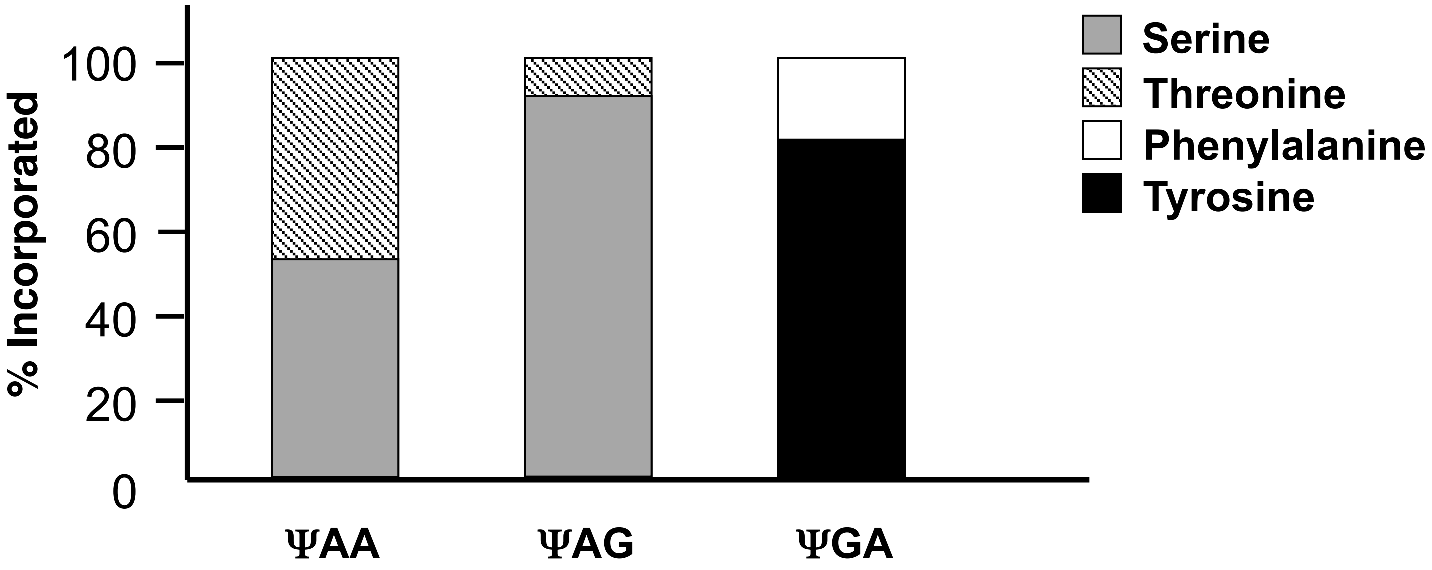
Trm4p F602TGA
w/ Specific Guide





#	b	b^{++}	b^0	b^{0++}	Seq.	y	y^{++}	y^*	y^{*++}	y^0	y^{0++}	#
1	114.0913	57.5493			L							12
2	201.1234	101.0653	183.1128	92.0600	S	**1214.5470**	607.7771	1197.5205	599.2639	1196.5364	598.7719	11
3	288.1554	144.5813	**270.1448**	135.5761	S	**1127.5150**	564.2611	1110.4884	**555.7479**	1109.5044	**555.2558**	10
4	345.1769	173.0921	327.1663	164.0868	G	**1040.4830**	520.7451	1023.4564	512.2318	1022.4724	511.7398	9
5	505.2075	253.1074	487.1969	244.1021	C	**983.4615**	**492.2344**	966.4349	483.7211	965.4509	483.2291	8
6	576.2446	288.6259	558.2341	279.6207	A	**823.4308**	412.2191	806.4043	403.7058	805.4203	403.2138	7
7	**739.3080**	370.1576	721.2974	**361.1523**	Y	**752.3937**	**376.7005**	735.3672	368.1872	734.3832	367.6952	6
8	**852.3920**	426.6996	834.3815	417.6944	I	**589.3304**	295.1688	572.3039	286.6556	571.3198	286.1636	5
9	**967.4190**	484.2131	949.4084	475.2078	D	**476.2463**	238.6268	459.2198	230.1135	458.2358	229.6215	4
10	**1066.4874**	533.7473	1048.4768	524.7420	V	**361.2194**	181.1133	344.1928	172.6001	343.2088	172.1081	3
11	**1153.5194**	577.2633	1135.5088	568.2581	S	**262.1510**	131.5791	245.1244	123.0659	244.1404	122.5738	2
12					R	175.1190	88.0631	158.0924	79.5498			1

Sup. Fig. 7. Identification of amino acids encoded at pseudouridylated UGA (ΨGA). (a) MS/MS spectrum (top) and mass of all possible ions associated with the phenylalanine-containing peptide (bottom). (b) MS/MS spectrum (top) and mass of all possible ions associated with the tyrosine-containing peptide (bottom). Bold masses represent ions identified.



Sup. Fig. 8. Quantification of the amino acids incorporated at pseudouridylated nonsense codons.

Nonsense Codon	Amino Acid	tRNA Anticodons			
ΨAA and ΨAG	Ser	AGA	CGA	TGA	GCT
	Thr	AGT	CGT	TGT	
ΨGA	Tyr	GTA			
	Phe	GAA			

Sup. Fig. 9. Schematic depiction of the tRNA anticodon sequences responsible for incorporating amino acids at pseudouridylated nonsense codons.

H/ACA RNA	Target ORF	Function of ORF
Known Guide Regions		
snR003	YKL050C	Unknown
snR031	YHR156C	U5 snRNP component
snR044	YPL060W	Mitochondria Mg^{2+} transporter
snR049	YJL060W	Kynurenine aminotransferase
snR080	YPL227C	Glucosyltransferase
snR081	YNL232W	RNA Exosome
Orphan Guide Regions		
snR009	YMR006C	Phospholipase B
snR011	YDL111C	RNA Exosome
snR030	YLR035C	DNA repair
	YAL047C	Microtubule nucleation
	YDR175C	Mitochondrial ribosomal protein

Sup Fig. 10. Computationally predicted endogenous nonsense codons that are targets of the H/ACA RNP machinery.