

Supplemental Material

The RES complex is required for efficient transformation of the pre-catalytic B spliceosome into an activated B^{act} complex

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Supplemental Figure 1

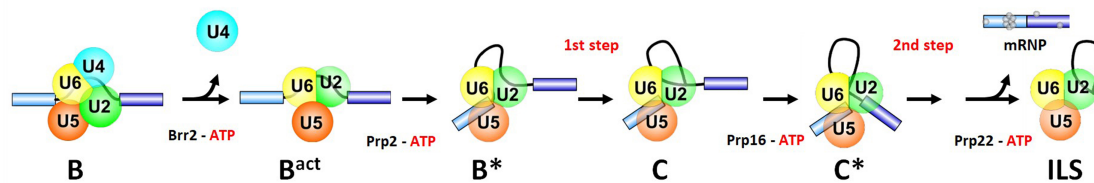


Figure S1 Spliceosome assembly pathway from the B complex to the ILS.

Supplemental Figure 2

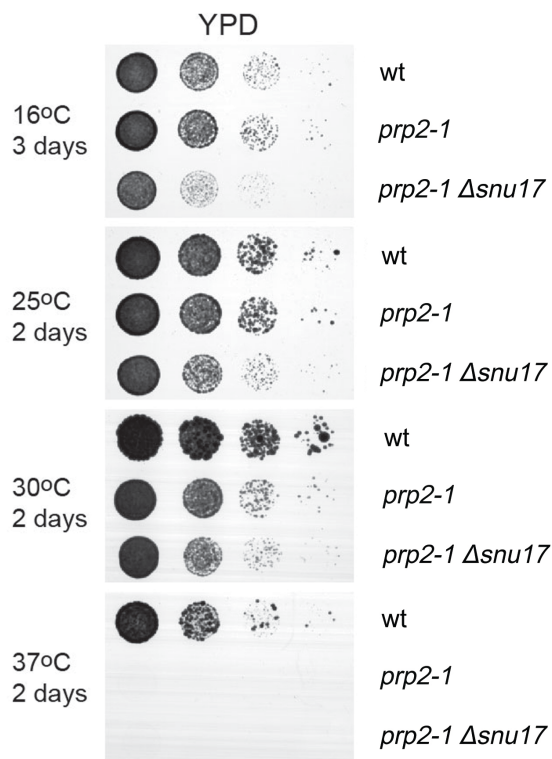


Figure S2 Comparison of the growth of *prp2-1* and *prp2-1 Δsnu17* yeast strains at different temperatures. Yeast cultures were grown to saturation at 25°C and each strain was adjusted to a culture density of 0.4 at 600 nm. Serial 10-fold dilutions (rows from left to right) were spotted onto agar medium and incubated for 2 or 3 days at 16, 25, 30 or 37°C, as indicated.

Supplemental Figure 3

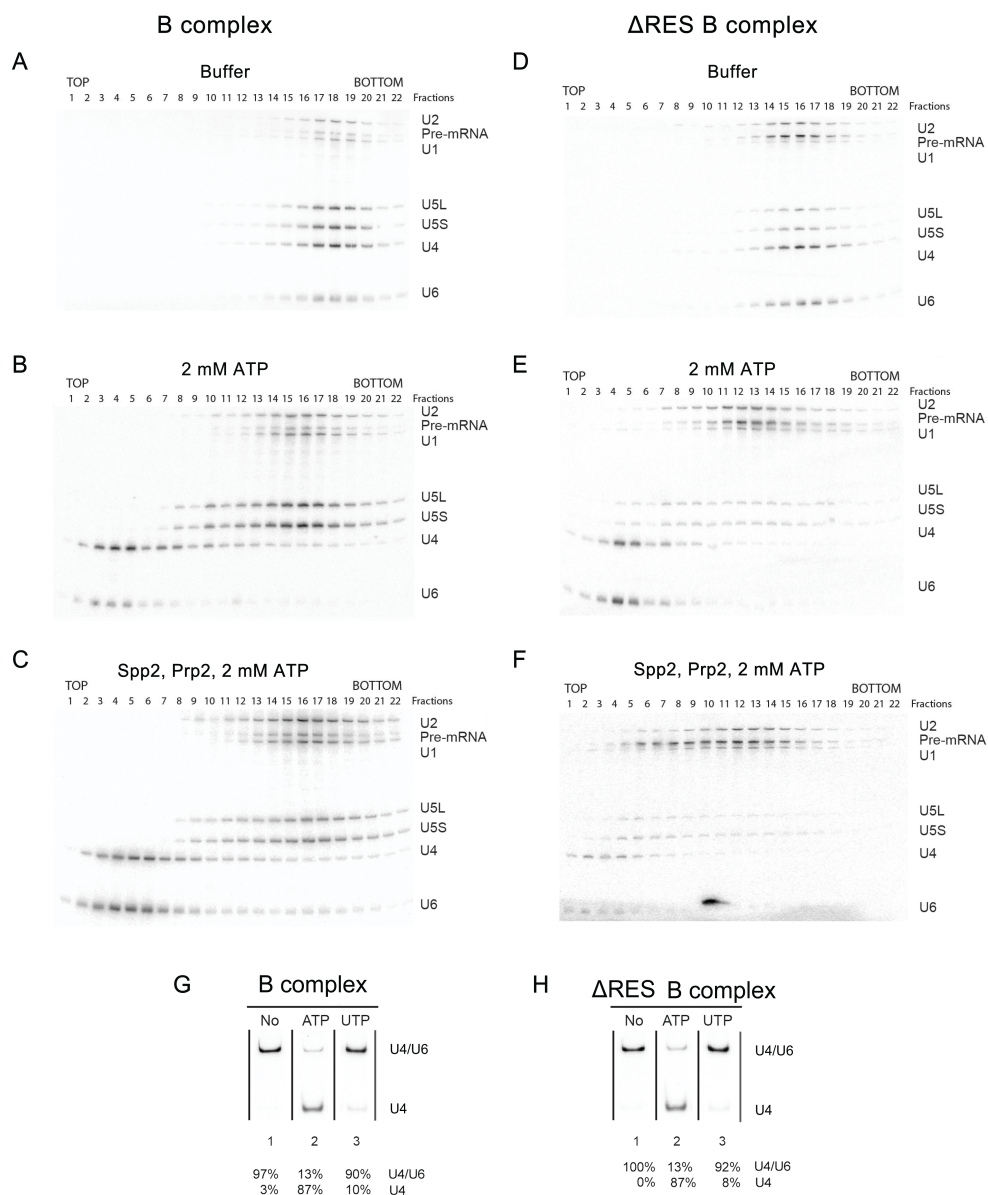


Figure S3 Brr2 unwinds U4/U6 in affinity-purified B complexes in a RES-independent manner. B complexes formed at 50 μ M ATP in yeast extract from a *prp2-1* strain (but without heat inactivation of Prp2) containing Snu17 (A-C) or lacking Snu17 (Δ RES) (D-F) were affinity purified, and subjected to a second glycerol gradient after incubation with (A, D) buffer, (B, E) 2 mM ATP or (C, F) ATP, Prp2 and Spp2. RNA was isolated and detected by Northern blotting with 32 P-labelled probes against the indicated snRNAs. The 32 P-actin pre-mRNA was detected by autoradiography. (G, H) RNA was isolated from B complexes formed in yeast extracts (G) with or (H) without Snu17, and analysed by native PAGE. U4 was visualized by Northern blotting.

Supplemental Figure 4

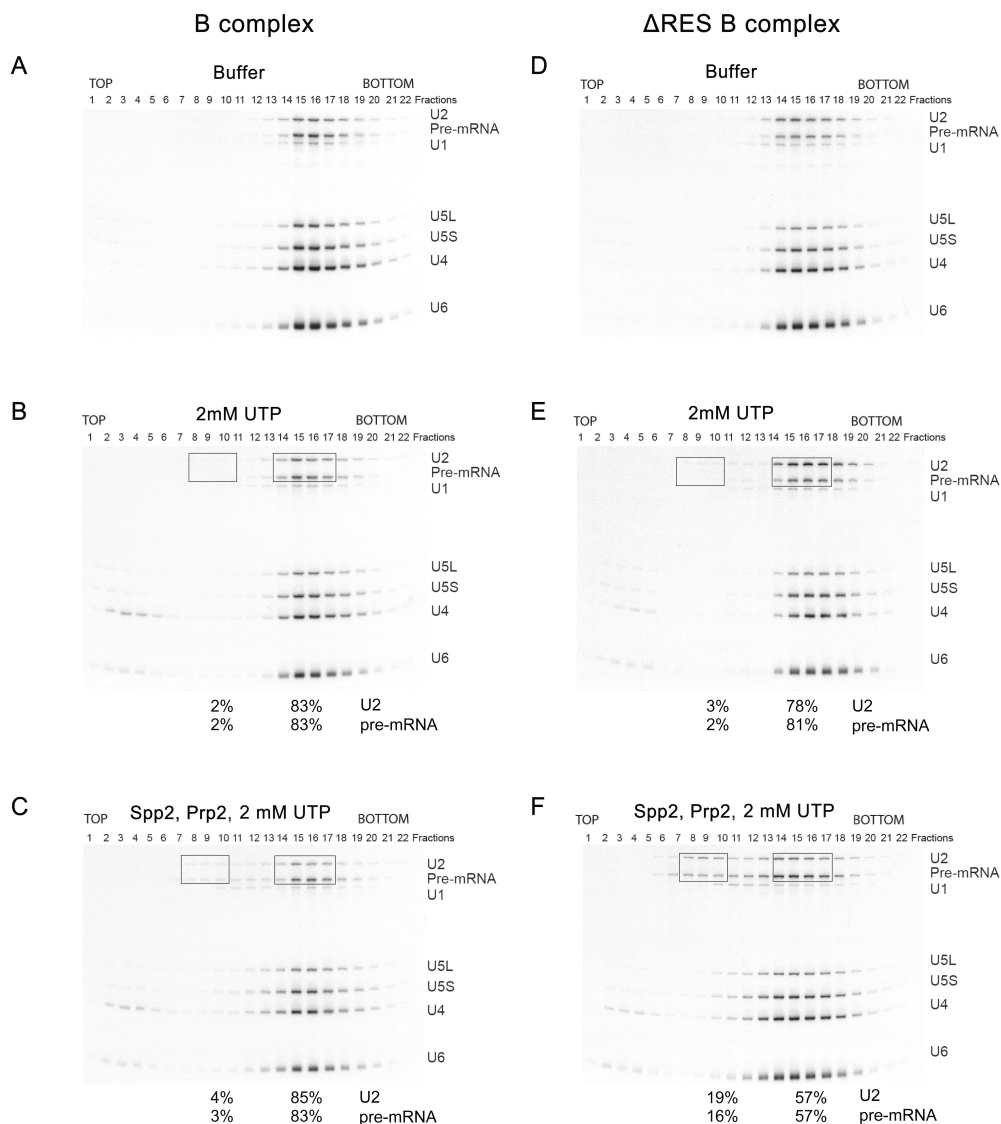


Figure S4 Prp2 RNA helicase facilitates the disassembly of purified ΔRES B complexes. B complexes formed in the presence of 50 μM ATP in yeast extracts from a *prp2-1* strain (but without heat inactivation of Prp2) containing (A-C) or lacking Snu17 (Δ*snu17*) (D-F) were affinity purified, and subjected to a second glycerol gradient after incubation with (A, D) buffer, (B, E) 2 mM UTP or (C, F) UTP, Prp2 and Spp2. RNA was isolated from each gradient fraction, separated by denaturing PAGE and detected by Northern blotting with ³²P-labelled probes against the snRNAs indicated on the right. The ³²P-labelled actin pre-mRNA was detected by autoradiography. The % of the U2 snRNA and pre-mRNA in the boxed fractions is shown below in selected panels.

Supplemental Figure 5

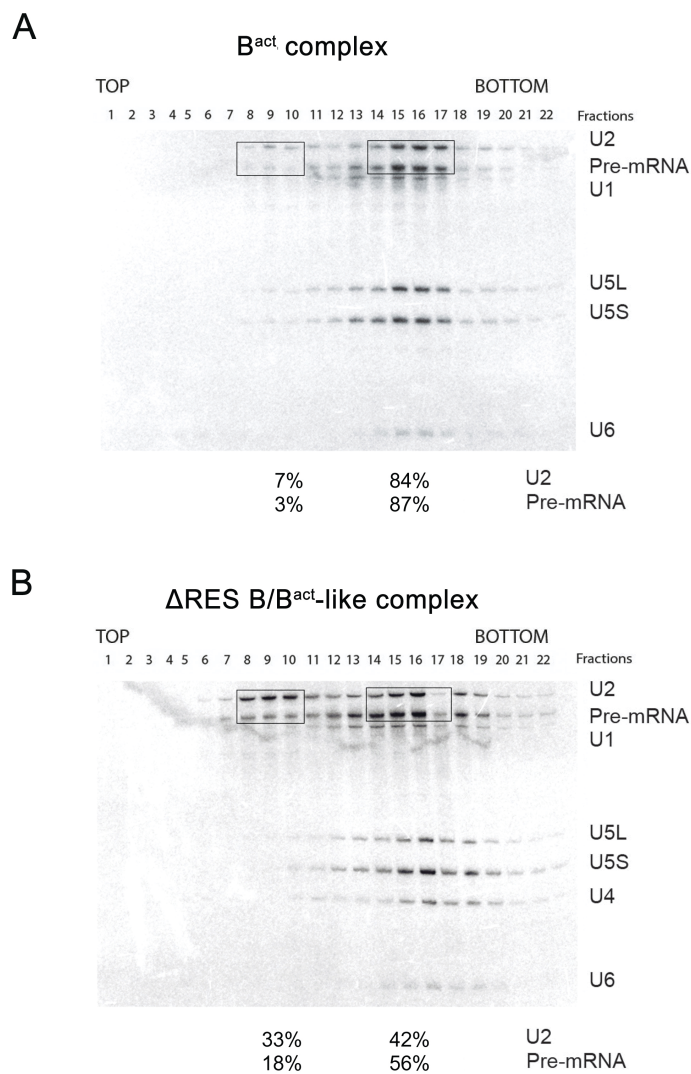


Figure S5 Affinity-purified B^{act} complexes containing RES are not susceptible to disassembly upon addition of Prp2, Spp2 and UTP. (A) B^{act} complexes formed in heat treated yeast extracts from a *prp2-1* strain or (B) B/B^{act}-like complexes formed in heat treated yeast extract from a *prp2-1 Δsnu17* strain were affinity purified, and subjected to a second glycerol gradient after incubation with UTP, Prp2 and Spp2. RNA was analysed as described in the legend to Figure S3.

Supplemental Figure 6

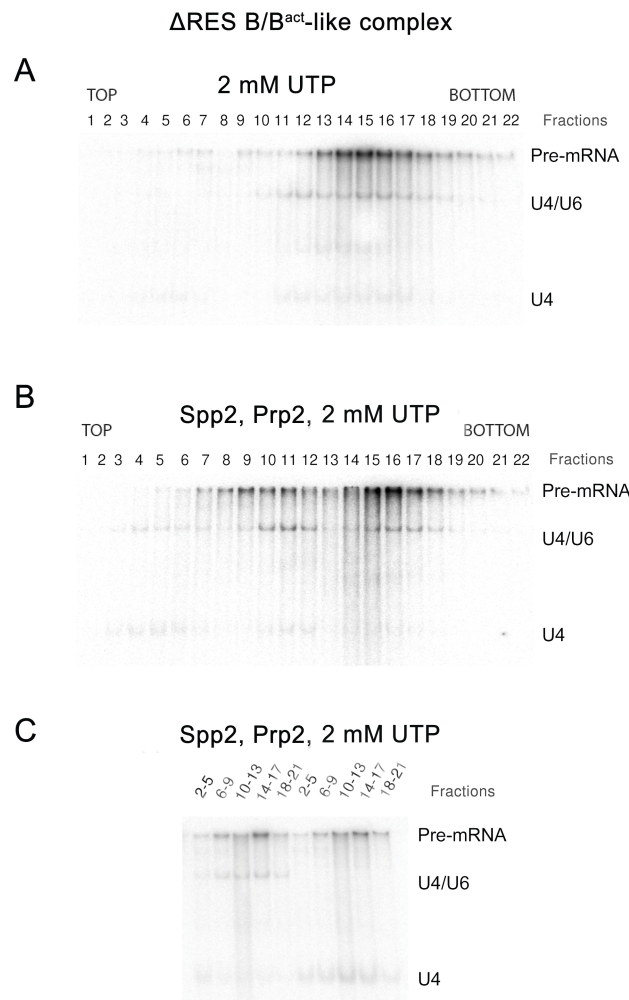


Figure S6 Analysis of U4/U6 duplex formation after incubation of ΔRES spliceosomes with UTP alone or UTP, Prp2 and Spp2. Affinity-purified ΔRES B/B^{act}-like complexes were subjected to a second glycerol gradient after incubation with (A) 2 mM UTP or (B, C) UTP, Prp2 and Spp2. In panels A and B, RNA was isolated from each gradient fraction and analysed by native PAGE. In panel C, RNA was isolated from three pooled gradient fractions (as indicated above) and either analysed directly by denaturing PAGE (left half) or after incubation at 95°C to disrupt U4/U6 duplexes (right half). In all panels, free U4 snRNA or U4 base paired with U6 was subsequently visualized by Northern blotting with a ³²P-probe complementary to U4 snRNA.

Supplemental Figure 7

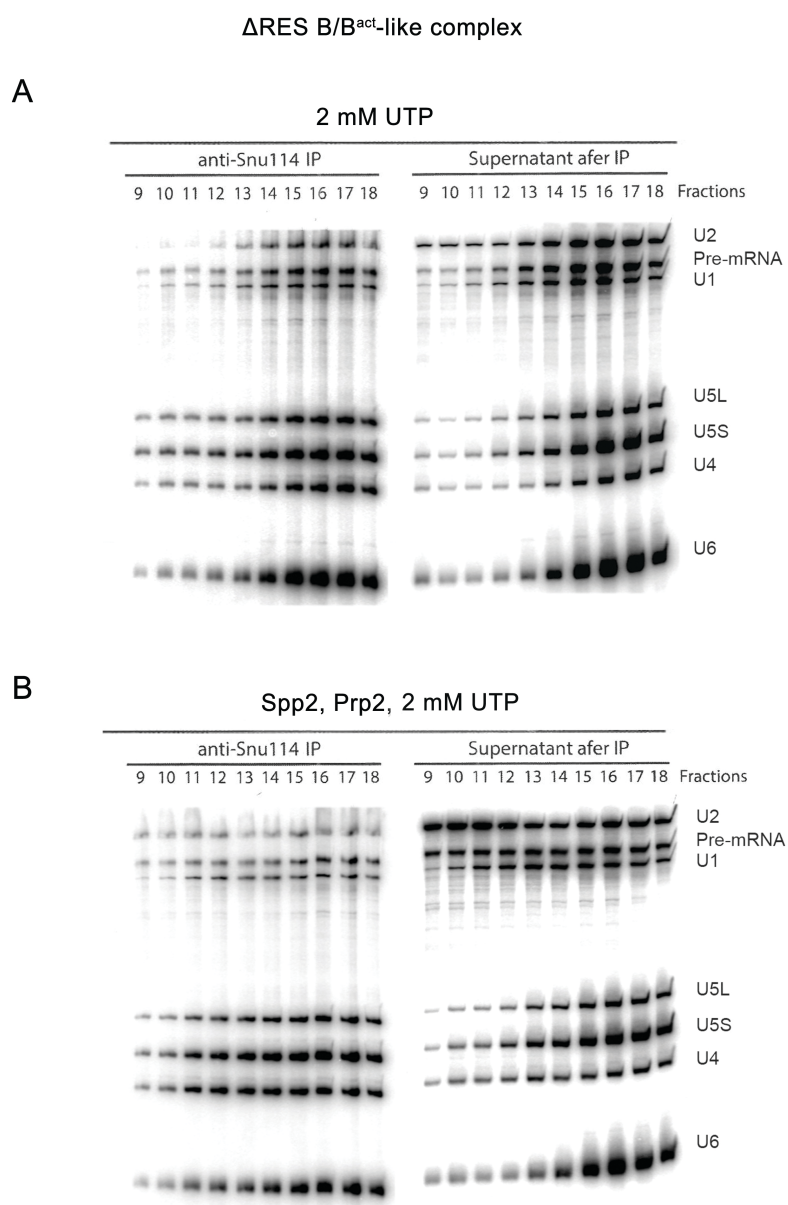


Figure S7 Immunoprecipitation of Snu114 containing complexes after incubation of Δ RES spliceosomes with UTP alone or UTP, Prp2 and Spp2. Affinity purified Δ RES B/B^{act}-like complexes were subjected to a second glycerol gradient after incubation with (A, B) UTP or (C, D) UTP, Prp2 and Spp2. RNP complexes in gradient fractions 9-18 were immunoprecipitated with antibodies against the U5 and U4/U6.U5 tri-snRNP protein Snu114. RNA coprecipitating together with Snu114, as well as RNA remaining in the supernatant after immunoprecipitation, was analysed by denaturing PAGE followed by Northern blotting (to visualize the snRNAs) or autoradiography (to detect the radiolabelled pre-mRNA).

Supplemental Figure 8

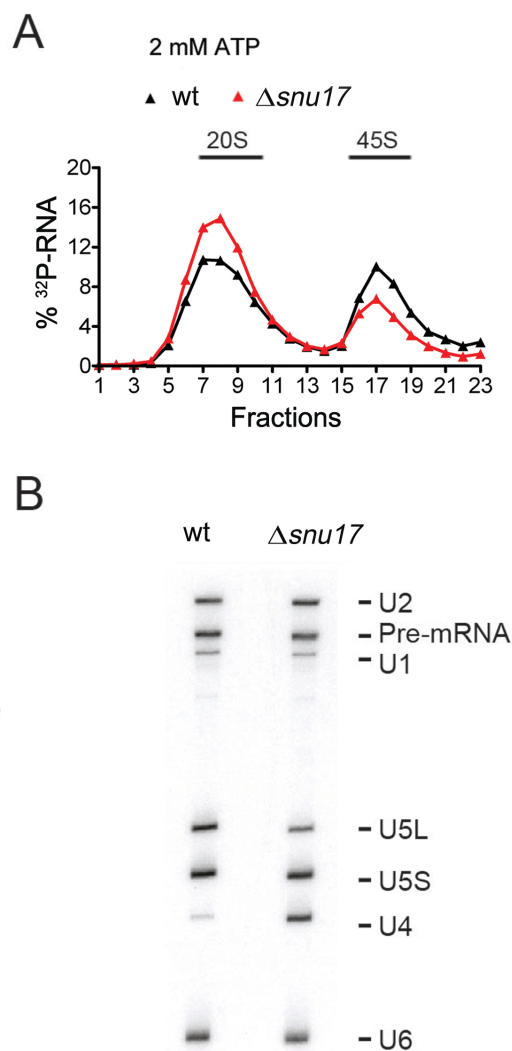


Figure S8 Characterization of spliceosomal complexes formed on a truncated Act Δ 6 pre-mRNA. (A) Glycerol gradient sedimentation profile of MS2-MBP affinity-purified spliceosomal complexes formed on ³²P-labelled Act Δ 6 pre-mRNA in extract from wt or Δ snu17 yeast cells after 60 min in the presence of 2 mM ATP. (B) RNA compositions of the affinity-purified complexes from the 45S peak fractions of the glycerol gradient. The pre-mRNA and snRNAs were separated by denaturing PAGE and detected as described in the legend to Figure S3.

Supplemental Methods

Yeast Growth Assays

To assay the growth phenotypes of the wildtype, *prp2-1* and *prp2-1 Δsnu17* strains, yeast cells were grown at 25°C in yeast extract/peptone medium (Formedium™) to an OD₆₀₀ of 4.0 and then collected by centrifugation. Yeast were then spotted after 10-fold serial dilutions on YPD plates and grown at 16°C , 25°C , 30°C or 37°C for 2-3 days.

Immunoprecipitations

Spliceosomal complexes were formed in extract from *prp2-1 Δsnu17* yeast cells (after heat inactivation of Prp2) and subsequently affinity purified and subjected to glycerol gradient centrifugation. Spliceosomes in the 45S gradient peak (Δ RES B/B^{act}-like complexes) were incubated with UTP, Prp2 and Spp2, and then analysed on a second glycerol gradient. For immunoprecipitation analysis, gradient fractions 9-18 were incubated with Protein A Sepharose bound by antibodies against the *S. cerevisiae* U5 Snu14 protein, with end-over-end rotation for 2 h. The supernatant was removed from the beads and the latter were 3X washed with NTN buffer [150 mM NaCl, 50 mM Tris-HCl, pH 7.5, 0.1% Nonidet P40 (v/v)]. Both the beads and supernatants were then digested with proteinase K, and the RNA was recovered by phenol extraction followed by ethanol precipitation. Purified RNAs were separated by 8% PAGE and analyzed by Northern blotting.

Tables S1-S5

Protein was recovered from the indicated complexes and analysed by LC-MSMS.

Numbers indicate the total number of peptides sequenced for a given protein.

Table S1. Protein composition of *S. cerevisiae* wt and *Asnu17* B spliceosomal complexes

Yeast name protein	Systematic gene name	MW (kDa)	wt	<i>Asnu17</i>
Sm proteins				
B	YER029C	22.4	97	113
D1	YGR074W	16.3	64	43
D2	YLR275W	12.8	67	85
D3	YLR147C	11.2	107	121
E	YOR159C	10.4	8	10
F or X3	YPR182W	9.6	13	14
G or X2	YFL017W-A	8.5	12	9
U1 snRNP				
Prp39	YML046W	74.7	137	84
Snu71	YGR013W	71.4	150	132
Prp40	YKL012W	69	145	143
Prp42	YDR235W	65	80	63
Mud2	YKL074C	60.5	39	46
Nam8	YHR086W	56.9	61	59
Snu56	YDR240C	56.5	63	57
Snp1	YIL061C	34.4	74	58
Mud1	YBR119W	34.4	48	59
Luc7	YDL087C	30	60	55
Yhc1	YLR298C	27	27	14
U2 snRNP				
Rse1	YML049C	153.8	612	534
Hsh155	YMR288W	110	350	361
Prp9	YDL030W	63	226	255
Cus1	YMR240C	50.2	173	185
Prp21	YJL203W	33	137	157
Prp11	YDL043C	29.9	91	90
Lea1	YPL213W	27.2	154	157
Hsh49	YOR319W	24.5	42	44
Msl1	YIR009W	12.8	41	45
Rds3	YPR094W	12.3	21	26
Ysf3	YNL138W-A	10	14	11
U5 snRNP				
Prp8	YHR165C	279.5	858	761
Brr2	YER172C	246.2	850	840
Snu114	YKL173W	114	459	489
Prp6	YBR055C	104.2	286	297
Prp28	YDR243C	66.6	0	2
Lin1	YHR156C	40.4	0	0
Dib1	YPR082C	16.7	39	34
U4/U6 snRNP				
Prp31	YGR091W	56.3	134	151
Prp3	YDR473C	56	164	202
Prp4	YPR178W	52.4	146	132
Snu13	YEL026W	13.6	19	12

U4/U6.U5 snRNP proteins				
Snu66	YOR308C	66.4	260	303
Sad1	YFR005C	52.2	0	0
Spp381	YBR152W	34	68	63
Prp38	YGR075C	28	71	84
Snu23	YDL098C	23	94	95
Lsm proteins				
Lsm4	YER112W	21.3	34	36
Lsm7	YNL147W	13	28	30
Lsm8	YJR022W	12.4	30	21
Lsm2	YBL026W	11.2	56	58
Lsm5	YER146W	10.4	9	11
Lsm3	YLR438C	10	2	2
Lsm6	YDR378C	9.4	20	18
RES complex				
Bud13	YGL174W	30.5	123	42
Pml1	YLR016C	23.6	79	0
Snu17/Ist3	YIR005W	17	35	0
NTC/Prp19 complex				
Syf1	YDR416W	100	186	157
Clf1	YLR117C	82.4	129	87
Cef1	YMR213W	68	169	129
Prp19	YLL036C	56.6	354	240
Isy1	YJR050W	28	51	41
Syf2	YGR129W	25	46	38
Snt309	YPR101W	21	40	31
Ntc20	YBR188C	16	29	23
NTC-related proteins				
Prp46	YPL151C	51	126	118
Prp45	YAL032C	42.5	125	115
Ecm2	YBR065C	41	53	50
Cwc2	YDL209C	38.4	74	33
Cwc15	YDR163W	20	20	28
Bud31	YCR063W	18.4	49	28
Early splicing factors				
Prp5	YBR237W	96.4	28	31
Urn1	YPR152C	54	169	126
Known splicing factors				
Prp2	YNR011C	100	1	16
Spp2	YOR148C	20.6	0	5
Yju2	YKL095W	32	35	14
Cwc21	YDR482C	15.7	18	29
Cwc22	YGR278W	67.3	3	4
Cwc24	YLR323C	28	1	1

Cwc27	YPL064C	35	3	2
Cwc23	YGL128C	33.2	0	0
Cwc25	YNL245C	20.4	0	1
Step 2 proteins				
Prp17	YDR364C	52	0	0
Prp22	YER013W	130	79	48
Prp16	YKR086W	121.6	0	2
Slu7	YDR088C	44.6	0	0
Prp18	YGR006W	28.4	0	0
Disassembly proteins				
Prp43	YGL120C	87.6	18	19
Spp382	YLR424W	83	0	0
Ntr2	YKR022C	36.6	0	0
CBP proteins				
Sto1	YMR125W	100	20	14
Cbc2	YPL178W	24	8	10

Table S2. Protein composition of *S. cerevisiae* *prp2-1* B^{act} and *prp2-1 Δsnu17* spliceosomes

Yeast name protein	Systematic gene name	MW (kDa)	<i>prp2-1</i>	<i>prp2-1 Δsnu17</i>
Sm proteins				
B	YER029C	22.4	140	160
D1	YGR074W	16.3	69	59
D2	YLR275W	12.8	87	90
D3	YLR147C	11.2	118	139
E	YOR159C	10.4	12	7
F or X3	YPR182W	9.6	16	17
G or X2	YFL017W-A	8.5	12	15
U1 snRNP				
Prp39	YML046W	74.7	167	205
Snu71	YGR013W	71.4	172	239
Prp40	YKL012W	69	175	260
Prp42	YDR235W	65	184	205
Mud2	YKL074C	60.5	67	63
Nam8	YHR086W	56.9	70	95
Snu56	YDR240C	56.5	68	108
Snp1	YIL061C	34.4	102	135
Mud1	YBR119W	34.4	77	107
Luc7	YDL087C	30	41	43
Yhc1	YLR298C	27	37	46
U2 snRNP				
Rse1	YML049C	153.8	1049	961
Hsh155	YMR288W	110	716	658
Prp9	YDL030W	63	409	394
Cus1	YMR240C	50.2	311	282
Prp21	YJL203W	33	248	211
Prp11	YDL043C	29.9	169	157
Lea1	YPL213W	27.2	243	190
Hsh49	YOR319W	24.5	75	82
Msl1	YIR009W	12.8	58	55
Rds3	YPR094W	12.3	42	30
Ysf3	YNL138W-A	10	21	24
U5 snRNP				
Prp8	YHR165C	279.5	1349	940
Brr2	YER172C	246.2	1774	1252
Snu114	YKL173W	114	986	708
Prp6	YBR055C	104.2	69	280
Prp28	YDR243C	66.6	7	2
Lin1	YHR156C	40.4	0	0
Dib1	YPR082C	16.7	14	40
U4/U6 snRNP				
Prp31	YGR091W	56.3	35	158
Prp3	YDR473C	56	62	171
Prp4	YPR178W	52.4	51	144
Snu13	YEL026W	13.6	5	19

U4/U6.U5 snRNP proteins				
Snu66	YOR308C	66.4	212	307
Sad1	YFR005C	52.2	0	0
Spp381	YBR152W	34	103	56
Prp38	YGR075C	28	133	66
Snu23	YDL098C	23	150	69
Lsm proteins				
Lsm4	YER112W	21.3	77	55
Lsm7	YNL147W	13	56	16
Lsm8	YJR022W	12.4	28	20
Lsm2	YBL026W	11.2	94	82
Lsm5	YER146W	10.4	25	16
Lsm3	YLR438C	10	11	6
Lsm6	YDR378C	9.4	32	25
RES complex				
Bud13	YGL174W	30.5	185	24
Pml1	YLR016C	23.6	126	0
Snu17/Ist3	YIR005W	17	54	0
NTC/Prp19 complex				
Syf1	YDR416W	100	245	226
Clf1	YLR117C	82.4	133	140
Cef1	YMR213W	68	173	163
Prp19	YLL036C	56.6	486	455
Isy1	YJR050W	28	66	65
Syf2	YGR129W	25	61	53
Snt309	YPR101W	21	63	52
Ntc20	YBR188C	16	31	34
NTC-related proteins				
Prp46	YPL151C	51	151	158
Prp45	YAL032C	42.5	174	174
Ecm2	YBR065C	41	183	87
Cwc2	YDL209C	38.4	101	113
Cwc15	YDR163W	20	125	66
Bud31	YCR063W	18.4	81	41
Early splicing factors				
Prp5	YBR237W	96.4	75	254
Urn1	YPR152C	54	280	131
Known splicing factors				
Prp2	YNR011C	100	0	0
Spp2	YOR148C	20.6	22	15
Yju2	YKL095W	32	101	68
Cwc21	YDR482C	15.7	41	46
Cwc22	YGR278W	67.3	104	99
Cwc24	YLR323C	28	81	46

Cwc27	YPL064C	35	73	9
Cwc23	YGL128C	33.2	2	16
Cwc25	YNL245C	20.4	1	13
Step 2 proteins				
Prp17/CDC40	YDR364C	52	69	78
Prp22	YER013W	130	77	121
Prp16	YKR086W	121.6	153	36
Slu7	YDR088C	44.6	0	114
Prp18	YGR006W	28.4	1	71
Disassembly proteins				
Prp43	YGL120C	87.6	19	77
Spp382	YLR424W	83	12	35
Ntr2	YKR022C	36.6	0	22
CBP proteins				
Sto1	YMR125W	100	20	69
Cbc2	YPL178W	24	9	23

Table S3 Distribution of proteins across the gradient after addition of 2 mM UTP to ΔRES B/B^{ect}-like spliceosomes.

Yeast name protein	Systematic gene name	MW (kDa)	Fractions 1-2	Fractions 3-4	Fractions 5-6	Fractions 7-8	Fractions 9-10	Fractions 11-12	Fractions 13-14	Fractions 15-16	Fractions 17-18	Fractions 19-20
Sm proteins												
B	YER029C	22.4	0	2	0	1	1	4	9	35	52	1
D1	YGR074W	16.3	0	1	0	2	4	6	14	37	51	18
D2	YLR275W	12.8	1	4	5	9	9	14	13	36	65	18
D3	YLR147C	11.2	2	6	7	10	13	22	21	57	107	35
E	YOR159C	10.4	0	0	0	1	0	0	3	6	13	5
F or X3	YPR182W	9.6	0	0	1	1	3	3	2	8	18	7
G or X2	YFL017W-A	8.5	0	0	1	2	1	3	3	10	15	3
U1 snRNP												
Prp39	YML046W	74.7	1	1	0	1	3	9	37	81	110	28
Snu71	YGR013W	71.4	0	1	1	1	3	24	52	73	112	42
Prp40	YKL012W	69	0	0	1	1	7	17	46	98	126	42
Prp42	YDR235W	65	0	0	1	0	3	12	42	82	115	30
Mud2	YKL074C	60.5	0	0	2	4	6	7	24	26	24	4
Nam8	YHR086W	56.9	0	0	0	0	2	7	13	26	47	16
Snu56	YDR240C	56.5	0	0	0	0	0	1	15	37	54	6
Snp1	YIL061C	34.4	1	0	0	0	0	6	21	33	65	14
Mud1	YBR119W	34.0	0	1	0	0	2	13	15	30	35	15
Luc7	YDL087C	30	3	2	1	1	2	11	10	16	20	13
Yhc1	YLR298C	27	0	1	0	1	0	1	2	7	9	2
U2 snRNP												
Rse1	YML049C	153.8	2	1	1	17	61	86	174	426	578	212
Hsh155	YMR288W	110	7	1	1	7	32	35	64	198	319	120
Prp9	YDL030W	63	2	2	1	3	23	30	47	126	220	68
Cus1	YMR240C	50.2	2	2	2	1	11	19	32	87	141	38
Prp21	YJL203W	33	3	1	0	4	5	11	33	94	146	34
Prp11	YDL043C	29.9	0	0	0	4	2	13	29	60	103	21
Lea1	YPL213W	27.2	4	6	6	10	10	22	24	78	113	9
Hsh49	YOR319W	24.5	1	1	1	3	2	4	4	15	28	1
Msl1	YIR009W	12.8	1	3	2	3	7	5	6	20	29	6
Rds3	YPR094W	12.3	1	3	0	1	4	6	6	13	15	4
Ysf3	YNL138W-A	10	0	0	0	2	3	4	4	14	18	10
U5 snRNP												
Prp8	YHR165C	279.5	1	1	1	6	62	36	166	471	650	215
Brr2	YER172C	246.2	5	5	17	46	111	134	303	531	771	262
Snu114	YKL173W	114	3	3	2	12	23	30	90	239	437	144
Prp6	YBR055C	104.2	2	5	13	16	11	8	22	94	216	60
Prp28	YDR243C	66.6										
Lin1	YHR156C	40.4			1							
Dib1	YPR082C	16.7	2	7	1	1	0	0	0	8	13	5
U4/U6 snRNP												
Prp31	YGR091W	56.3	0	1	7	8	6	7	8	42	81	21
Prp3	YDR473C	56	1	0	4	6	7	5	12	49	97	17
Prp4	YPR178W	52.4	0	3	11	17	12	19	23	57	72	24
Snu13	YEL026W	13.6	4	1	0	2	4	3	2	7	14	5
U4/U6.U5 snRNP proteins												
Snu66	YOR308C	66.4	0	0	1	2	7	16	28	89	163	64
Sad1	YFR005C	52.2	1	1	0	0	1	0	0	0	0	0
Spp381	YBR152W	34	2	0	5	2	4	3	5	20	28	6
Prp38	YGR075C	28	3	7	7	6	2	10	9	38	57	6
Snu23	YDL098C	23	1	3	3	6	1	4	4	19	22	0
Lsm proteins												
Lsm4	YER112W	21.3	0	1	4	5	1	3	3	15	28	7
Lsm7	YNL147W	13	1	1	1	2	2	3	2	6	17	2
Lsm8	YJR022W	12.4	1	3	3	5	2	3	2	6	11	2
Lsm2	YBL026W	11.2	1	10	8	19	7	13	10	28	51	14
Lsm5	YER146W	10.4	1	3	1	3	2	2	2	7	5	2
Lsm3	YLR438C	10	0	0	0	0	0	0	0	1	2	1
Lsm6	YDR378C	9.4	0	5	3	5	3	5	7	8	11	8
RES complex												
Bud13	YGL174W	30.5	0	0	0	1	1	3	3	10	4	0
Pml1	YLR016C	23.6	0	0	0	1	1	0	0	0	0	0
Snu17/Ist3	YIR005W	17										
NTC/Prp19 complex												
Syf1	YDR416W	100	1	0	0	0	0	9	43	132	203	59
Cif1	YLR117C	82.4	1	1	0	0	1	8	31	58	107	46
Cef1	YMR213W	68	0	0	0	0	0	7	35	76	123	54
Prp19	YLL036C	56.6	0	2	6	9	10	38	128	264	385	134
Isy1	YJR050W	28	0	0	0	0	1	2	6	20	35	6
Syf2	YGR129W	25	0	0	0	0	0	7	11	33	40	14
Snt309	YPR101W	21	0	0	0	1	1	1	3	6	10	3
Ntc20	YBR188C	16	1	0	0	0	1	2	3	11	9	3
NTC-related proteins												
Prp46	YPL151C	51	0	0	0	1	0	9	23	86	153	26
Prp45	YAL032C	42.5	1	2	4	0	2	6	21	96	158	32
Ecm2	YBR065C	41	0	1	1	0	0	0	0	9	22	0
Cwc2	YDL209C	38.4	3	2	0	2	0	6	11	46	81	21
Cwc15	YDR163W	20	1	3	1	12	2	8	11	45	59	2

Bud31	YCR063W	18.4	0	0	0	0	2	4	15	23	35	12
Early splicing factors												
Prp5	YBR237W	96.4	0	1	1	5	14	13	44	89	81	19
Urn1	YPR152C	54	0	3	9	17	20	26	26	26	38	8
Known splicing factors												
Prp2	YNR011C	100	2	1	0	3	0	0	0	1	1	1
Spp2	YOR148C	20.6	0	0	1	0	1	5	1	7	9	0
Yju2	YKL095W	32	0	0	1	0	1	8	10	43	54	12
Cwc21	YDR482C	15.7	0	3	1	6	5	11	12	28	34	17
Cwc22	YGR278W	67.3	0	0	0	0	2	0	14	38	92	29
Cwc24	YLR323C	28	0	3	3	7	3	4	2	7	16	5
Cwc27	YPL064C	35	0	0	0	2	1	1	2	1	1	2
Cwc23	YGL128C	33.2	0	0	0	0	0	2	3	3	7	2
Cwc25	YNL245C	20.4	0	0	0	1	0	0	0	5	6	0
Step 2 proteins												
Prp17	YDR364C	52	1	1	1	0	0	1	8	37	54	25
Prp22	YER013W	130	0	1	1	1	3	1	21	68	89	24
Prp16	YKR086W	121.6	4	0	1	1	1	0	3	5	16	1
Slu7	YDR088C	44.6	1	1	0	3	9	14	15	35	42	8
Prp18	YGR006W	28.4	0	0	1	1	5	14	15	17	21	5
Disassembly proteins												
Prp43	YGL120C	87.6	4	14	14	5	1	9	30	30	28	5
Spp382	YLR424W	83	0	1	1	1	2	6	16	15	26	5
Ntr2	YKR022C	36.6	0	0	0	0	3	2	10	4	6	1
CBP proteins												
Sto1	YMR125W	100	3	3	4	7	2	1	7	16	16	1
Cbc2	YPL178W	24	1	4	4	9	1	2	0	1	3	0

Table S4 Distribution of proteins across the gradient after addition of UTP, Prp2 and Spp2 to Δ RES B/B^{ect}-like spliceosomes.

Yeast name protein	Systematic gene name	MW (kDa)	Fractions 1-2	Fractions 3-4	Fractions 5-6	Fractions 7-8	Fractions 9-10	Fractions 11-12	Fractions 13-14	Fractions 15-16	Fractions 17-18	Fractions 19-20
Sm proteins												
B	YER029C	22.4	5	2	3	9	6	15	15	23	14	5
D1	YGR074W	16.3	0	2	3	8	8	16	6	13	9	4
D2	YLR275W	12.8	0	2	5	10	17	22	19	23	15	7
D3	YLR147C	11.2	0	3	6	19	21	28	22	33	22	12
E	YOR159C	10.4	1	0	1	1	1	3	1	2	1	0
F or X3	YPR182W	9.6	0	1	1	2	2	1	3	2	2	2
G or X2	YFL017W-A	8.5	1	1	1	1	3	6	8	7	4	2
U1 snRNP												
Prp39	YML046W	74.7	2	0	2	2	7	29	34	33	26	13
Snu71	YGR013W	71.4	1	2	2	1	7	54	33	41	42	14
Prp40	YKL012W	69	3	0	0	2	16	46	51	44	51	16
Prp42	YDR235W	65	0	0	0	0	3	27	28	34	37	11
Mud2	YKL074C	60.5	1	0	2	2	6	18	12	10	4	2
Nam8	YHR086W	56.9	0	0	0	1	4	24	11	16	15	9
Snu56	YDR240C	56.5	0	0	0	1	2	16	19	23	21	1
Snp1	YIL061C	34.4	2	0	0	0	3	16	8	13	11	2
Mud1	YBR119W	34.4	2	0	0	1	3	21	12	16	14	10
Luc7	YDL087C	30	1	1	0	3	3	19	9	9	11	8
Yhc1	YLR298C	27	0	0	0	0	0	2	3	7	4	1
U2 snRNP												
Rse1	YML049C	153.8	6	1	2	49	220	246	138	224	209	64
Hsh155	YMR288W	110	1	0	0	23	81	124	70	134	104	26
Prp9	YDL030W	63	3	0	3	21	71	81	54	85	80	40
Cus1	YMR240C	50.2	1	0	0	12	31	51	32	61	39	13
Prp21	YJL203W	33	1	0	0	9	21	34	13	30	22	9
Prp11	YDL043C	29.9	0	0	0	8	16	25	10	25	18	8
Lea1	YPL213W	27.2	5	9	5	26	35	52	27	50	37	17
Hsh49	YOR319W	24.5	1	1	0	6	3	5	6	8	6	3
Msl1	YIR009W	12.8	1	2	1	9	7	17	5	8	9	3
Rds3	YPR094W	12.3	4	2	1	7	9	14	7	8	4	4
Ysf3	YNL138W-A	10	0	0	1	4	6	4	4	5	2	2
U5 snRNP												
Prp8	YHR165C	279.5	4	0	0	2	48	117	178	269	244	83
Brr2	YER172C	246.2	10	2	32	33	98	222	223	327	266	91
Snu114	YKL173W	114	1	0	0	6	31	77	109	218	175	54
Prp6	YBR055C	104.2	5	12	14	6	13	34	52	48	47	8
Prp28	YDR243C	66.6										
Lin1	YHR156C	40.4										
Dib1	YPR082C	16.7	3	3	0	0	0	3	3	4	3	2
U4/U6 snRNP												
Prp31	YGR091W	56.3	2	1	2	2	5	18	20	24	25	12
Prp3	YDR473C	56	1	5	9	11	7	15	20	37	25	8
Prp4	YPR178W	52.4	0	3	8	11	8	29	25	33	32	13
Snu13	YEL026W	13.6	2	0	0	4	0	4	4	4	2	2
U4/U6.U5 snRNP proteins												
Snu66	YOR308C	66.4	1	0	1	4	17	52	39	61	61	15
Sad1	YFR005C	52.2										1
Spp381	YBR152W	34	4	1	3	0	3	9	5	6	3	0
Prp38	YGR075C	28	1	4	3	4	7	21	13	20	18	5
Snu23	YDL098C	23	2	2	3	1	4	8	11	9	7	5
Lsm proteins												
Lsm4	YER112W	21.3	0	1	4	6	5	7	6	10	12	3
Lsm7	YNL147W	13	0	2	1	3	2	2	1	1	1	1
Lsm8	YJR022W	12.4	2	4	3	2	3	2	6	4	3	2
Lsm2	YBL026W	11.2	0	8	12	17	9	18	11	21	12	7
Lsm5	YER146W	10.4	2	1	3	3	1	6	5	2	4	3
Lsm3	YLR438C	10	0	0	0	1	0	0	0	0	0	0
Lsm6	YDR378C	9.4	2	2	7	4	5	6	8	8	7	1
RES complex												
Bud13	YGL174W	30.5	2	0	0	0	0	1	2	0	0	0
Pml1	YLR016C	23.6										
Snu17/Ist3	YIR005W	17										
NTC/Prp19 complex												
Syf1	YDR416W	100	1	0	3	1	2	22	53	115	96	32
Cfl1	YLR117C	82.4	3	0	1	2	1	12	23	53	36	8
Cef1	YMR213W	68	1	0	0	0	0	10	25	61	43	19
Prp19	YLL036C	56.6	3	7	5	5	7	53	101	203	164	57
Isy1	YJR050W	28	0	0	0	0	0	3	4	8	7	2
Syf2	YGR129W	25	1	0	0	0	0	8	11	26	19	11
Snt309	YPR101W	21	2	0	0	0	0	0	0	5	4	3
Ntc20	YBR188C	16	0	0	0	0	0	0	2	5	3	2
NTC-related proteins												
Prp46	YPL151C	51	0	0	0	0	0	10	28	59	45	17
Prp45	YAL032C	42.5	1	0	0	0	0	14	21	51	34	14
Ecm2	YBR065C	41	2	0	0	0	0	0	4	4	3	0
Cwc2	YDL209C	38.4	0	0	0	0	0	4	10	23	21	5
Cwc15	YDR163W	20	1	4	5	5	4	11	11	32	23	10
Bud31	YCR063W	18.4	1	0	0	3	1	2	8	12	13	5
Early splicing												

factor													
Prp5	YBR237W	96.4	2	0	0	6	17	40	38	42	18	5	
Urn1	YPR152C	54	2	13	20	19	23	24	19	22	10	3	
Known splicing factors													
Prp2	YNR011C	100	336	822	780	287	181	94	48	61	51	30	
Spp2	YOR148C	20.6	242	163	138	74	37	26	10	9	0	0	
Yju2	YKL095W	32	0	0	0	0	0	5	13	21	15	8	
Cwc21	YDR482C	15.7	1	3	2	3	3	8	10	17	12	7	
Cwc22	YGR278W	67.3	1	10	8	0	0	2	15	39	38	14	
Cwc24	YLR323C	28	2	8	10	4	1	1	0	1	1	0	
Cwc27	YPL064C	35	1	1	2	0	0	0	0	0	0	0	
Cwc23	YGL128C	33.2	0	0	0	1	0	4	3	4	3	1	
Cwc25	YNL245C	20.4	2	0	0	0	0	0	0	2	2	0	
Step 2 proteins													
Prp17	YDR364C	52	1	1	1	1	0	4	13	24	20	5	
Prp22	YER013W	130	6	0	1	1	0	1	16	45	33	11	
Prp16	YKR086W	121.6	1	0	0	0	0	0	2	13	9	2	
Slu7	YDR088C	44.6	7	0	0	8	11	18	24	33	20	10	
Prp18	YGR006W	28.4	1	0	0	0	2	6	9	13	5	2	
Disassembly proteins													
Prp43	YGL120C	87.6	21	34	35	13	12	11	19	17	11	5	
Spp382	YLR424W	83	0	0	0	1	1	6	17	19	7	2	
Ntr2	YKR022C	36.6	0	0	0	0	1	3	6	2	4	2	
CBP proteins													
Sto1	YMR125W	100	3	15	18	16	7	3	7	6	3	0	
Cbc2	YPL178W	24	3	2	4	3	1	2	1	5	1	1	

Table S5 Protein composition of *S. cerevisiae prp2-1 Δsnu17* spliceosomes formed on ActΔ6 pre-mRNA

Yeast name protein	Systematic gene name	MW (kDa)	<i>prp2-1</i>	<i>prp2-1 Δsnu17</i>
Sm proteins				
B	YER029C	22.4	63	76
D1	YGR074W	16.3	46	42
D2	YLR275W	12.8	55	74
D3	YLR147C	11.2	91	83
E	YOR159C	10.4	15	10
F or X3	YPR182W	9.6	9	7
G or X2	YFL017W-A	8.5	12	11
U1 snRNP				
Prp39	YML046W	74.7	69	79
Snu71	YGR013W	71.4	55	85
Prp40	YKL012W	69	53	74
Prp42	YDR235W	65	48	50
Mud2	YKL074C	60.5	42	42
Nam8	YHR086W	56.9	19	29
Snu56	YDR240C	56.5	31	52
Snp1	YIL061C	34.4	32	37
Mud1	YBR119W	34.4	19	19
Luc7	YDL087C	30	28	26
Yhc1	YLR298C	27	13	14
U2 snRNP				
Rse1	YML049C	153.8	677	571
Hsh155	YMR288W	110	448	448
Prp9	YDL030W	63	256	272
Cus1	YMR240C	50.2	189	256
Prp21	YJL203W	33	138	134
Prp11	YDL043C	29.9	92	90
Lea1	YPL213W	27.2	129	123
Hsh49	YOR319W	24.5	61	36
Msl1	YIR009W	12.8	47	37
Rds3	YPR094W	12.3	27	18
Ysf3	YNL138W-A	10	14	10
U5 snRNP				
Prp8	YHR165C	279.5	904	785
Brr2	YER172C	246.2	1021	931
Snu114	YKL173W	114	437	492
Prp6	YBR055C	104.2	72	182
Prp28	YDR243C	66.6	0	2
Lin1	YHR156C	40.4	0	0
Dib1	YPR082C	16.7	11	11
U4/U6 snRNP				
Prp31	YGR091W	56.3	29	78
Prp3	YDR473C	56	29	68
Prp4	YPR178W	52.4	27	82

Snu13	YEL026W	13.6	3	6
U4/U6.U5 snRNP proteins				
Snu66	YOR308C	66.4	66	168
Sad1	YFR005C	52.2	0	0
Spp381	YBR152W	34	8	22
Prp38	YGR075C	28	19	39
Snu23	YDL098C	23	29	48
Lsm proteins				
Lsm4	YER112W	21.3	24	28
Lsm7	YNL147W	13	10	9
Lsm8	YJR022W	12.4	12	10
Lsm2	YBL026W	11.2	30	26
Lsm5	YER146W	10.4	6	10
Lsm3	YLR438C	10	3	3
Lsm6	YDR378C	9.4	12	13
RES complex				
Bud13	YGL174W	30.5	106	29
Pml1	YLR016C	23.6	67	0
Snu17/Ist3	YIR005W	17	37	0
NTC/Prp19 complex				
Syf1	YDR416W	100	339	309
Clf1	YLR117C	82.4	251	230
Cef1	YMR213W	68	234	237
Prp19	YLL036C	56.6	507	441
Isy1	YJR050W	28	80	83
Syf2	YGR129W	25	70	61
Snt309	YPR101W	21	78	38
Ntc20	YBR188C	16	28	23
NTC-related proteins				
Prp46	YPL151C	51	186	221
Prp45	YAL032C	42.5	209	229
Ecm2	YBR065C	41	125	104
Cwc2	YDL209C	38.4	173	133
Cwc15	YDR163W	20	120	119
Bud31	YCR063W	18.4	64	37
Early splicing factors				
Prp5	YBR237W	96.4	68	77
Urn1	YPR152C	54	62	82
Known splicing factor				
Prp2	YNR011C	100	412	360
Spp2	YOR148C	20.6	76	78
Yju2	YKL095W	32	120	109
Cwc21	YDR482C	15.7	50	56
Cwc22	YGR278W	67.3	176	155

Cwc24	YLR323C	28	90	96
Cwc27	YPL064C	35	132	75
Cwc23	YGL128C	33.2	2	8
Cwc25	YNL245C	20.4	2	3
Step 2 proteins				
Prp17	YDR364C	52	116	103
Prp22	YER013W	130	78	168
Prp16	YKR086W	121.6	16	38
Slu7	YDR088C	44.6	3	60
Prp18	YGR006W	28.4	3	46
Disassembly proteins				
Prp43	YGL120C	87.6	51	125
Spp382	YLR424W	83	3	28
Ntr2	YKR022C	36.6	2	8
CBP proteins				
Sto1	YMR125W	100	28	18
Cbc2	YPL178W	24	7	3