

Supplemental Table S1. Yeast strains used in this study.

Name	Genotype	Description	Source or Reference
yAAH1930	MATa ade2 cup1Δ::ura3 his3 leu2 lys2 trp1 ura3 GAL prp22Δ::loxP p360-22 (Prp22 URA CEN ARS)	Cu ²⁺ -sensitive shuffle strain for Prp22. yMK-02	Charles Query/Magda Konarska
yAAH1931	yAAH1930 + pAAH1042	Prp22 ^{WT}	Charles Query/Magda Konarska
yAAH1932	yAAH1930 + pAAH1043	Prp22 ^{T637A}	Charles Query/Magda Konarska
yAAH1937	yAAH1930 + ECM2::hphMX	Cu ²⁺ -sensitive, ecm2Δ shuffle strain for Prp22	This work.
yAAH1943	yAAH1937 + pAAH1042	Prp22 ^{WT} , ecm2Δ	This work.
yAAH2482	yAAH1937 + pAAH1043	Prp22 ^{T637A} , ecm2Δ	This work.
yAAH2044	yAAH1943 + pAAH0137	Prp22 ^{WT} , ecm2 Δ, empty URA3 plasmid	This work.
yAAH2045	yAAH1943 + pAAH1056	Prp22 ^{WT} , ecm2 Δ, (ECM2 ^{WT} URA3 CEN ARS)	This work.
yAAH2047	yAAH1943 + pAAH1067	Prp22 ^{WT} , ecm2 Δ, (ECM2 ¹⁻¹⁴³ URA3 CEN ARS)	This work.
yAAH2047	yAAH1943 + pAAH1068	Prp22 ^{WT} , ecm2 Δ, (ECM2 ¹⁻¹⁹⁷ URA3 CEN ARS)	This work.
yAAH2047	yAAH1943 + pAAH1196	Prp22 ^{WT} , ecm2 Δ, (ECM2 ¹⁻²⁶⁵ URA3 CEN ARS)	This work.
yAAH2047	yAAH1943 + pAAH1197	Prp22 ^{WT} , ecm2 Δ, (ECM2 ¹⁻³²⁵ URA3 CEN ARS)	This work.
yAAH2257	MATα cup1Δ ura3 his3 trp1 lys2 ade2 leu2 hsh155::kanMX prp2::hphMX ecm2::natMX + (HSH155 TRP1 CEN ARS) + (PRP2 URA3 CEN ARS)	Cu ²⁺ -sensitive, ecm2Δ shuffle strain for Prp2	This work.
yAAH2258	yAAH2257 + pAAH0778	Prp2 ^{WT} , ecm2Δ	This work.
yAAH2259	yAAH2257 + pAAH0790	Prp2 ^{Q548N} , ecm2Δ	This work.
yAAH1915	MATα cup1Δ ura3 his3 trp1 lys2 ade2 leu2 hsh155::kanMX prp2::hphMX + (HSH155 TRP1 CEN ARS) + (PRP2 ^{WT} LEU2 CEN ARS)	Prp2 ^{WT}	(1)
yAAH1916	MATα cup1Δ ura3 his3 trp1 lys2 ade2 leu2 hsh155::kanMX prp2::hphMX + (HSH155 TRP1 CEN ARS) + (PRP2 ^{Q548N} LEU2 CEN ARS)	Prp2 ^{Q548N}	(1)
yAAH0130	MATa trp1 ura3 his lys2 leu2 ade2 prp16D::lys2 pSB2-Prp16 (PRP16 URA)	shuffle strain for Prp16, yS78	Christine Guthrie (2)
yAAH1936	yAAH0130 + ECM2::hphMX	ecm2Δ shuffle strain for Prp16	This work.
yAAH1946	yAAH0130 + pAAH1040	Prp16 ^{WT}	This work.
yAAH1947	yAAH0130 + pAAH1039	Prp16 ^{R686I}	This work.
yAAH1941	yAAH1936 + pAAH1040	Prp16 ^{WT} , ecm2Δ	This work.
yAAH1942	yAAH1936 + pAAH1039	Prp16 ^{R686I} , ecm2Δ	This work.
yAAH0434	MATα cup1Δ ura3 his3 trp1 lys2 ade2 leu2	Cu ²⁺ -sensitive strain	David Brow
yAAH1691	yAAH0434 + ECM2::hphMX	Cu ²⁺ -sensitive, ecm2Δ strain	This work.
yAAH2490	yAAH2435+pAAH0470	Prp8 ^{WT} Cu ²⁺ -sensitive strain with WT ACT-CUP1	This work.

yAAH2491	yAAH2446+pAAH0470	Prp8 ^{WT} Cu ²⁺ -sensitive, ecm2Δ strain with WT ACT-CUP1	This work.
yAAH2492	yAAH2435+pAAH1032	Prp8 ^{WT} Cu ²⁺ -sensitive strain with A3C ACT-CUP1	This work.
yAAH2493	yAAH2446+pAAH1032	Prp8 ^{WT} Cu ²⁺ -sensitive, ecm2Δ strain with A3C ACT-CUP1	This work.
yAAH2494	yAAH2435+pAAH0514	Prp8 ^{WT} Cu ²⁺ -sensitive strain with A3U ACT-CUP1	This work.
yAAH2495	yAAH2446+pAAH0514	Prp8 ^{WT} Cu ²⁺ -sensitive, ecm2Δ strain with A3U ACT-CUP1	This work.
yAAH2496	yAAH2435+pAAH0517	Prp8 ^{WT} Cu ²⁺ -sensitive strain with G5A ACT-CUP1	This work.
yAAH2497	yAAH2446+pAAH0517	Prp8 ^{WT} Cu ²⁺ -sensitive, ecm2Δ strain with G5A ACT-CUP1	This work.
yAAH2498	yAAH2435+pAAH1058	Prp8 ^{WT} Cu ²⁺ -sensitive strain with cryptic 5'SS ACT1-CUP1	This work.
yAAH2499	yAAH2446+pAAH1058	Prp8 ^{WT} Cu ²⁺ -sensitive, ecm2Δ strain with cryptic 5' SS ACT-CUP1	This work.
yAH2500	yAAH2435+pAAH0439	Prp8 ^{WT} Cu ²⁺ -sensitive strain with U257C ACT-CUP1	This work.
yAH2501	yAAH2446+pAAH0439	Prp8 ^{WT} Cu ²⁺ -sensitive, ecm2Δ strain with U257C ACT-CUP1	This work.
yAH2502	yAAH2435+pAAH0524	Prp8 ^{WT} Cu ²⁺ -sensitive strain with A258U ACT-CUP1	This work.
yAH2503	yAAH2446+pAAH0524	Prp8 ^{WT} Cu ²⁺ -sensitive, ecm2Δ strain with A258U ACT-CUP1	This work.
yAH2504	yAAH2435+pAAH0441	Prp8 ^{WT} Cu ²⁺ -sensitive strain with A259C, BS-C ACT-CUP1	This work.
yAH2505	yAAH2446+pAAH0441	Prp8 ^{WT} Cu ²⁺ -sensitive, ecm2Δ strain with A259C, BS-C ACT-CUP1	This work.
yAH2506	yAAH2435+pAAH0880	Prp8 ^{WT} Cu ²⁺ -sensitive strain with A259G, BS-G ACT-CUP1	This work.
yAH2507	yAAH2446+pAAH0880	Prp8 ^{WT} Cu ²⁺ -sensitive, ecm2Δ strain with A259G, BS-G ACT-CUP1	This work.
yAH2508	yAAH2435+pAAH0527	Prp8 ^{WT} Cu ²⁺ -sensitive strain with A302U ACT-CUP1	This work.
yAH2509	yAAH2446+pAAH0527	Prp8 ^{WT} Cu ²⁺ -sensitive, ecm2Δ strain with A302U ACT-CUP1	This work.
yAAH2449	yAAH1911 + pAAH1015	U2-WT	This work.
yAAH2455	yAAH1913 + pAAH1015	U2-WT, ecm2Δ	This work.
yAAH2453	yAAH1911 + pAAH1020	U2-Ilc+	This work.
yAAH2460	yAAH1913 + pAAH1020	U2-Ilc+, ecm2Δ	This work.
yAAH2452	yAAH1911 + pAAH1018	U2-2,4	This work.
yAAH2458	yAAH1913 + pAAH1018	U2-2,4, ecm2Δ	This work.
yAAH2451	yAAH1911 + pAAH1017	U2-G53A	This work.
yAAH2457	yAAH1913 + pAAH1017	U2-G53A, ecm2Δ	This work.
yAAH2453	yAAH1911 + pAAH1019	U2-ΔIIb	This work.

yAAH2459	yAAH1913 + pAAH1019	U2- Δ IIb, ecm2 Δ	This work.
yAAH2450	yAAH1911 + pAAH1016	U2- Δ CC	This work.
yAAH2456	yAAH1913 + pAAH1016	U2- Δ CC, ecm2 Δ	This work.
yAAH1911	U2 shuffle MATa ade2 cup1 Δ ::ura3 his3 leu2 lys2 prp5 Δ ::loxP trp1 snr20 Δ ::loxP (pRS314-PRP5 (PRP5 TRP1 CEN ARS) pRS316-U2 (SNR20 URA3 CEN ARS))	Cu ²⁺ -sensitive shuffle strain for U2 and Prp5, yZX11.	(3)
yAAH1913	yAAH1911 + ECM2::hphMX	Cu ²⁺ -sensitive shuffle strain for U2 and Prp5, ecm2 Δ	This work.
yAAH0117	MATa, cup1 Δ ura3 his3 leu2 lys2 prp8 Δ ::lys2 trp1 pJU169 (PRP8 URA3)	Cu ²⁺ -sensitive shuffle strain for Prp8, yJU75	Christine Guthrie (4)
yAAH1886	yAAH0117 + ECM2::hphMX	Cu ²⁺ -sensitive shuffle strain for Prp8, ecm2 Δ	This work.
yAAH2435	yAAH0117 + pAAH0997	Prp8 ^{WT}	This work.
yAAH2446	yAAH1886 + pAAH0997	Prp8 ^{WT} , ecm2 Δ	This work.
yAAH2439	yAAH0117 + pAAH1045	Prp8 ^{V1870N} (<i>prp8-162</i>)	This work.
yAAH2440	yAAH1886 + pAAH1045	Prp8 ^{V1870N} (<i>prp8-162</i>), ecm2 Δ	This work.
yAAH2441	yAAH0117 + pAAH1003	Prp8 ^{E1960K} (<i>prp8-101</i>)	This work.
yAAH2442	yAAH1886 + pAAH1003	Prp8 ^{E1960K} (<i>prp8-101</i>), ecm2 Δ	This work.
yAAH2437	yAAH0117 + pAAH1001	Prp8 ^{P986T} (<i>prp8-161</i>)	This work.
yAAH2438	yAAH1886 + pAAH1001	Prp8 ^{P986T} (<i>prp8-161</i>), ecm2 Δ	This work.
yAAH2443	yAAH0117 + pAAH1004	Prp8 ^{R1753K}	This work.
yAAH2444	yAAH1886 + pAAH1004	Prp8 ^{R1753K} , ecm2 Δ	This work.
yAAH2445	yAAH0117 + pAAH1006	Prp8 ^{P986T/R1753K}	This work.
yAAH2446	yAAH1886 + pAAH1006	Prp8 ^{P986T/R1753K} , ecm2 Δ	This work.
yAAH2447	yAAH0117 + pAAH1007	Prp8 ^{E1960K/V1870N}	This work.
yAAH2448	yAAH1886 + pAAH1007	Prp8 ^{E1960K/V1870N} , ecm2 Δ	This work.
yAAH1908	yAAH0434 + snr6::natMX + pRS316-U4U6mini (SNR4 SNR6 URA3)	Cu ²⁺ -sensitive shuffle strain for U6 snRNA	This work.
yAAH1910	yAAH1908 + ECM2::hphMX	Cu ²⁺ -sensitive shuffle strain for U6 snRNA, ecm2 Δ	This work.
yAAH2434	yAAH1910 + pAAH0137 + pAAH0412	WT U6 + Empty URA	This work.
yAAH2433	yAAH1910 + pAAH1056 + pAAH0412	WT U6 + Ecm2 ^{WT}	This work.
yAAH2432	yAAH1910 + pAAH1067 + pAAH0412	WT U6 + Ecm2 ¹⁻¹⁴³	This work.
yAAH2476	yAAH1910 + pAAH0137 + pAAH1027	U57A U6 + Empty URA	This work.
yAAH2477	yAAH1910 + pAAH1056 + pAAH1027	U57A U6 + Ecm2 ^{WT}	This work.
yAAH2478	yAAH1910 + pAAH1067 + pAAH1027	U57A U6 + Ecm2 ¹⁻¹⁴³	This work.

yAAH2479	yAAH1910 + pAAH0137 + pAAH1028	U57C U6 + Empty URA	This work.
yAAH2480	yAAH1910 + pAAH1056 + pAAH1028	U57C U6 + Ecm2 ^{WT}	This work.
yAAH2481	yAAH1910 + pAAH1067 + pAAH1028	U57C U6 + Ecm2 ¹⁻¹⁴³	This work.

1. T. J. Carrocci, D. M. Zoerner, J. C. Paulson, A. A. Hoskins, SF3b1 mutations associated with myelodysplastic syndromes alter the fidelity of branchsite selection in yeast. *Nucleic Acids Res*, **45**, 4837-4852 (2017).
2. Y. Wang, C. Guthrie, PRP16, a DEAH-box RNA helicase, is recruited to the spliceosome primarily via its nonconserved N-terminal domain. *RNA (New York, NY)*. **4**, 1216–1229 (1998).
3. Y.-Z. Xu, C. C. Query, Competition between the ATPase Prp5 and branch region-U2 snRNA pairing modulates the fidelity of spliceosome assembly. *Molecular Cell*. **28**, 838–849 (2007).
4. J. G. Umen, C. Guthrie, A novel role for a U5 snRNP protein in 3' splice site selection. *Gene Dev*. **9**, 855–868 (1995).