

Identification and characterization of *Schizosaccharomyces pombe* splicing mutants

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Abstract

Pre-mRNA splicing is carried out by the spliceosome, a dynamic complex of five small nuclear ribonucleoprotein particles (snRNPs). Several genetic screens have been conducted in *Schizosaccharomyces pombe* to identify pre-mRNA splicing mutants and spliceosome components. However, some pre-mRNA splicing mutants have yet to be assigned to a gene and in certain cases, the mutations within genes have not been identified and phenotypes compared. Here, we have identified new mutations in the U4/U6.U5 tri-snRNP component [dim1](#) and assigned *prp6* and *prp7* mutants to [snu13](#) and [brl1](#), respectively, revealing roles for these factors in pre-mRNA splicing.

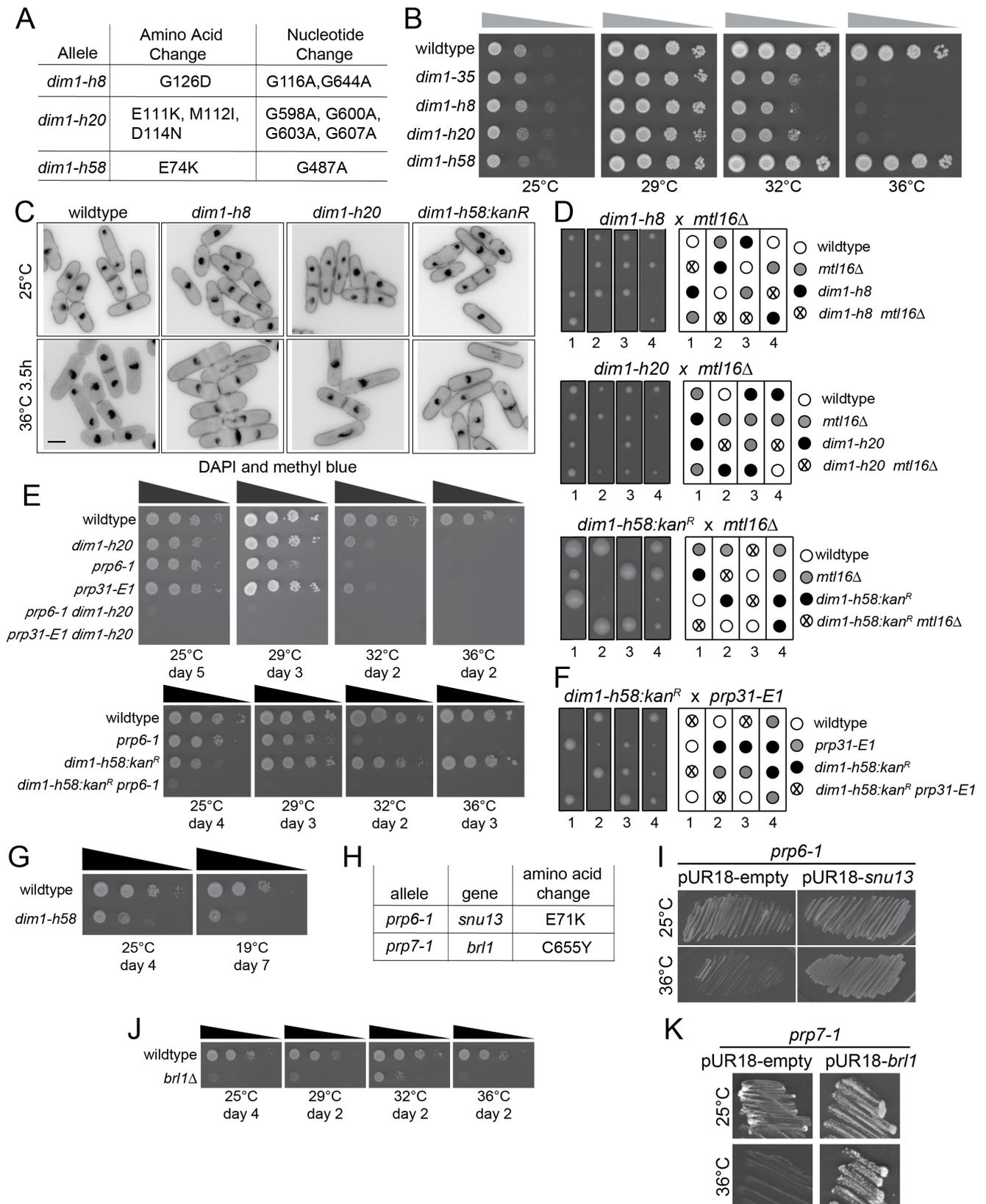


Figure 1. Sequence analysis of pre-mRNA splicing mutants.:

(A) The mutations encoded by each *dim1* allele are listed. (B, E, G, and J) The indicated strains were grown in liquid YE media at 25°C until they reached mid-log phase and then adjusted to OD = 0.20. Next, 10-fold serial dilutions were made and 2.5 μ L of each was spotted on YE agar plates and incubated at the indicated temperatures for 2-7 days prior to imaging. Images of all plates shown in panel B were acquired after less than 3 days of growth, accounting for the difference in wildtype growth at 25°C compared to other panels. (C) The indicated strains were grown at 25°C and shifted to 36°C for 3.5 hours. Samples were collected at both temperatures and cells were fixed and stained with DAPI and

Methyl Blue before imaging. (D and F) Representative tetrads incubated on YE plates at 25°C from the indicated cross and schematics of relevant genotypes. (H) The responsible gene and mutations encoded by each allele are listed. (I and K) *prp6-1* (I) or *prp7-1* (K) cells containing the indicated plasmids were streaked to the indicated temperatures and incubated for 3-5 days.

Description

Pre-mRNA splicing requires the activity of the spliceosome, a large and dynamic complex comprised of dozens of protein components (Wilkinson et al., 2020). In *S. pombe*, almost half of the ~5134 protein coding genes contain one or more introns in their pre-mRNAs that require spliceosome-mediated excision (Carme et al., 2026; Rutherford et al., 2024; Wood et al., 2012). This complexity which also includes the use of degenerate splice site sequences, exonic splicing enhancers, and SR proteins, makes *S. pombe* an attractive organism for the elucidation of pre-mRNA processing mechanisms (Fair and Pleiss, 2017).

[Dim1](#) is an essential, highly conserved component of the U4/U6.U5 tri-snRNP (Berry and Gould, 1997; Gottschalk et al., 1999; Reuter et al., 1999; Stevens and Abelson, 1999; Zhang et al., 2000; Zhang et al., 1999). In a genetic screen designed to identify genes cooperating with [Cdk1](#) to promote the G2/M transition, we isolated the *dim1-35* mutation because it reduced the restrictive temperature of *cdc2-D217N* (Berry and Gould, 1997). The *dim1-35* mutant, in which amino acid 126 is changed from G to D, is defective in pre-mRNA splicing (Carnahan et al., 2005), displays significant cell cycle defects (Berry and Gould, 1997), and is synthetically lethal with a mutant in the anaphase-promoting complex, *lid1-6* (Berry et al., 1999) and with loss of U6 snRNA m⁶A methyltransferase [Mtl16](#) function (Willet et al., 2023). In the same screen used to isolate *dim1-35*, three additional [dim1](#) mutant alleles were obtained but not characterized. Here, we determine their mutations and examine their phenotypes and genetic interactions. We also identify the causative gene mutations in two previously unassigned pre-mRNA splicing mutants.

To determine what mutations were present in the previously uncharacterized [dim1](#) alleles, the open reading frame was amplified from each strain and sequenced. The *dim1-h8* strain contained the same amino acid change as did *dim1-35* (Berry and Gould, 1997) plus a nucleotide change within the second intron that is not at an intron junction or the splicing branch point. *dim1-h20* contained four mutations leading to three amino acid changes and the *dim1-h58* strain contained a single mutation resulting in an E74K change (Figure 1A). The range of growth was determined for each strain by spotting at a variety of temperatures. The *dim1-h8* and *dim1-h20* mutants showed similar temperature-sensitivity to *dim1-35* and failed to form colonies at 36°C (Figure 1B). In contrast, *dim1-h58* was not heat-sensitive (Figure 1B) and therefore to follow it in crosses, the *kanMX6* cassette was inserted after the [dim1](#) stop codon. DAPI and Methyl Blue staining showed that *dim1-h8* resembled *dim1-35* at the non-permissive temperature with a variety of chromosome segregation errors including chromatin bisected by septa (Berry and Gould, 1997) (Figure 1C). *dim1-h20* and *dim1-h58:kanR* cells appeared a bit elongated at 36°C and the chromosomes were sometimes disorganized but cut cells were not observed (Figure 1C). Like *dim1-35* (Willet et al., 2023), all three previously uncharacterized [dim1](#) mutants were synthetically lethal with *mtl16Δ* (Figure 1D). Also like *dim1-35*, *dim1-h20* displayed significant negative genetic interactions with *prp6-1* and *prp31-E1* (Figure 1E). Interestingly, despite a lack of heat-sensitivity on its own, *dim1-h58* also displayed a significant negative genetic interaction with *prp6-1* (Figure 1E) and was synthetically lethal with *prp31-E1* at 25°C (Figure 1F). These genetic interactions prompted us to test whether *dim1-h58* was cold rather than heat sensitive. Indeed, we found that it was (Figure 1G).

Like [Dim1](#), [Prp31](#) is a component of the U4/U6.U5 tri-snRNP and both *dim1-35* and *prp31-E1* show significant negative genetic interactions with *prp6-1* (Bishop et al., 2000; Willet et al., 2023). Interestingly, neither *prp6-1* nor *prp7-1*, among the first *S. pombe* temperature-sensitive mutants defective in pre-mRNA processing identified (Potashkin et al., 1998; Potashkin et al., 1989; Urushiyama et al., 1996), have been assigned to a gene. To learn what gene mutations gave rise to these two *prp* mutants, we crossed each of the two strains to wildtype, grew 8 colonies of wildtype and 8 colonies of mutant obtained from crossing to our laboratory wildtype strain, combined the 8 samples of each at equal cell concentrations, and the genomes of the two samples were sequenced and analyzed. A comparison of the mutant sequences to that of wildtype indicated that the *prp6-1* strain carries an E71K substitution in the [snu13](#) open reading frame (G211A) (Figure 1H). [Snu13](#) is required for both pre-mRNA splicing and pre-rRNA splicing in *Saccharomyces cerevisiae* and is a tri-snRNP component (Dobbyn et al., 2007; Dobbyn and O'Keefe, 2004) that in *S. pombe* co-purifies with [Dim1](#) (Carnahan et al., 2005). Amplifying and sequencing the [snu13](#) gene from *prp6-1* cells confirmed the presence of this mutation. The [psc3](#) gene is immediately adjacent to [snu13](#) on chromosome I and we found that no wildtype progeny were recovered from a cross between *prp6-1* and *psc3-1T:kanR* (Nonaka et al., 2002) in a plate of tetrads. Further, plasmid-expressed [snu13](#)⁺ was able to rescue *prp6-1* temperature-sensitivity (Figure 1I). Taken together, we conclude that the [Snu13](#) E71K substitution is responsible for the *prp6-1* phenotype.

Two candidate mutations were identified in the *prp7-1* strain relative to wild type: *mdn1-D4125N* (G12373A) and *brl1-C655Y* (G1964A). We focused our attention on [brl1](#) because its homolog in *S. cerevisiae*, *BRE1*, has been linked to pre-mRNA splicing (Moehle et al., 2012) through its role in histone H2B ubiquitination (Herissant et al., 2014; Moehle et al.,

2012). In contrast, there is no evidence that [Mdn1](#) plays a role in pre-mRNA splicing. Amplifying and sequencing the [brl1](#) gene from *prp7-1* cells confirmed the presence of the *brl1-C655Y* mutation. The [bmt5](#) gene is closely linked to [brl1](#) on chromosome III (Lock et al., 2018) and we found no recombinants between *bmt5Δ::kanR* and *prp7-1* in 10 complete tetrads. Although *brl1Δ* is not an essential gene, *brl1Δ* cells were reported to be slow growing and highly elongated with an increased septation index (Tanny et al., 2007; Zofall and Grewal, 2007). A similar elongated, hyphal-like phenotype was also noted for *prp7-1* cells after shift to the non-permissive temperature (Potashkin et al., 1998). We verified that the *brl1Δ* strain grows slowly and also determined that it is unable to form colonies at all on YE plates at 36°C (Figure 1J). Finally, plasmid-expressed [brl1](#)⁺ was able to rescue the temperature-sensitivity and also the slow growth at 25°C of *prp7-1* (Figure 1K). We conclude that the *prp7-1* pre-mRNA processing phenotype is due to the mutation in [brl1](#).

In sum, our data provide evidence for the cooperation of [Dim1](#), [Prp31](#), and [Snu13](#) in spliceosome activation. Further, by identifying the mutations in the *prp6-1* and *prp7-1* strains we firmly link both [Snu13](#) and [Brl1](#) to the process of pre-mRNA splicing, enhancing the toolkit of reagents with which to investigate mechanisms of this critical process in *S. pombe*.

Methods

Yeast methods

S. pombe strains were grown in yeast extract (YE) or Edinburgh minimal medium (EMM) supplemented with appropriate amino acids with appropriate supplements and standard *S. pombe* mating, sporulation, and tetrad dissection techniques were used for backcrossing, outcrossing, and to construct new strains (Forsburg and Rhind, 2006; Moreno et al., 1991). EMM with 5 μg/ml thiamine was used to repress expression of murine *dim1* from the *nmt1* promoter.

To construct *dim1-h58::kanMX6*, PCR was utilized to amplify a sequence containing the kanamycin resistant gene in a pFA6 cassette (Bahler et al., 1998). Through lithium acetate transformation (Keeney and Boeke, 1994), this sequence was inserted after the final stop codon of the *dim1-h58* gene. Colonies were selected by replication onto YE plates containing G418 (Geneticin, 100 μg/mL, Thermo Fisher Scientific; cat# 11811031), and correct marking of the [dim1](#) allele was confirmed through whole-cell PCR.

For growth assays, strains were grown overnight in YE at 25°C to OD₅₉₅=0.1-0.8. Cells were then adjusted to OD₅₉₅ = 0.20 and then diluted tenfold thrice. 2.5 μL of each dilution were spotted onto YE plates and grown at the indicated temperatures for several days. Each growth assay was performed twice.

Molecular biology methods

A PCR product was generated from each [dim1](#) allele using an oligonucleotide 59 bp upstream of the start site (GTATATTGTGTGACTTACATATCTACA) and 243 bp downstream of the stop codon (GCAGTAATCATGTTTCATGC) (Integrated DNA technologies), purified, and sent for sequencing. To construct plasmids containing [brl1](#) and [snu13](#), PCR was used to amplify sequences of [brl1](#) and [snu13](#) from wildtype genomic DNA that included 300 bp flanks upstream and downstream of the coding sequence. These fragments were each cloned into the BamHI site of pUR18 (Barbet et al., 1992) using Gibson assembly (Gibson et al., 2009). Subsequently, the pUR18-[brl1](#) and pUR18-[snu13](#) plasmids were transformed into *prp7-1* and *prp6-1*, respectively, using the lithium acetate transformation method (Keeney and Boeke, 1994), and colonies were selected on EMM plates lacking uracil. PCR products, plasmids, and whole genomes were sequenced by Plasmidsaurus using Oxford Nanopore Technologies (ONT) long-read sequencing.

ONT read mapping and variant calling

Whole-genome ONT sequencing reads were down-sampled to 30x depth coverage using seqtk v1.5-r133 (<https://github.com/lh3/seqtk>). Down-sampled reads were mapped to the reference genome with minimap2 v2.30-r1287 (<https://github.com/lh3/minimap2>) (Li, 2018). Variants were called using Clair3 v1.2.0 (<https://github.com/HKU-BAL/Clair3>) (Zheng et al., 2022) with the r1041_e82_400bps_sup_v500 model. Clair3-generated gVCF files were merged using GLnexus v1.4.1 (<https://github.com/dnanexus-rnd/GLnexus>) (Yun et al., 2021). Variants were annotated using SnpEff v4_3t (<https://pcingola.github.io/SnpEff/>) (Cingolani et al., 2012). Candidate mutations were defined as variants predicted to cause amino acid changes that were present in the mutant sample but absent in the wild-type sample. Detailed scripts, commands, and parameters used in this analysis are available at (https://github.com/fsnibs10/prp6_prp7). Whole-genome ONT sequencing reads have been deposited in the Sequence Read Archive (SRA) of the National Center for Biotechnology Information (NCBI) under BioProject accession [PRJNA1428504](https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1428504).

Microscopy methods

To visualize nuclei and septa, cells were grown overnight in YE to log phase at 25°C, shifted to 36.5°C for 3.5 hours and fixed in ice-cold 70% ethanol. Samples were then washed in phosphate-buffered saline three times and stained with 1 mg/mL Methyl Blue (Millipore Sigma) and 4',6-diamidino-2-phenylindole (DAPI). Single medial Z slices were obtained using a Zeiss Axio Observer inverted epifluorescence microscope with an AxioCam 503 mono camera and a Zeiss Plan Apochromat 63x oil (1.46 NA) objective. Representative images were formatted using ImageJ (Schindelin et al., 2012).

Reagents

Strain	Genotype	Source
KGY246	<i>ura4-D18 leu1-32 ade6-M210 h⁻</i>	Lab stock
KGY247	<i>ura4-D18 leu1-32 ade6-M210 h⁺</i>	Lab stock
KGY390	<i>dim1-35 ura4-D18 leu1-32 h⁻</i>	Lab stock
KGY1152	<i>dim1-h8 ura4-D18 leu1-32 ade6-M21X h⁻</i>	This study
KGY5712-2	<i>dim1-h8 ade6-M210 ura4-D18 leu1-32 h⁺</i>	This study
KGY1153	<i>dim1-h20 ura4-D18 leu1-32 ade6-M21X h⁻</i>	This study
KGY1160	<i>dim1-h20 h⁻</i>	This study
KGY8680-2	<i>dim1-h58 ura4-D18 leu1-32 ade6-M210 h⁺</i>	This study
KGY7226-2	<i>dim1-h58:kanMX6 ura4-D18 leu1-32 ade6-M210 h⁻</i>	This study
KGY1301	<i>mtl16::ura4⁺ ura4-D18 leu1-32 ade6-M21X h⁻</i>	Willet et al., 2023
KGY1844	<i>mtl16::ura4⁺ ura4-D18 leu1-32 ade6-M21X h⁺</i>	Willet et al., 2023
KGY2457	<i>prp31-E1 ade6-M210 leu1-32 ura4-D18 h⁻</i>	Bishop et al., 2000
KGY1877	<i>prp6-1 leu1-32 h⁻</i>	Potashkin et al., 1998
KGY8434	<i>prp6-1 ura4-D18 h⁺</i>	This study
KGY8678-2	<i>dim1-h20 prp31-E1 ade6-M210 leu1-32 ura4-D18 h⁻</i>	This study
KGY8679-2	<i>dim1-h20 prp6-1 ade6-M210 leu1-32 ura4-D18 h⁻</i>	This study
KGY7045-2	<i>dim1-h58:kanMX6 prp6-1 ade6-M210 leu1-32 ura4-D18 h⁻</i>	This study
KGY7218	<i>prp7-1 leu1-32 h⁻</i>	Potashkin et al., 1998
KGY7591-2	<i>prp7-1 ura4-D18 leu1-32 Ade6? h[?]</i>	This study
KGY7671	<i>pcs3-1T:kanR ade6-M210 h⁻</i>	Nonaka et al., 2002
KGY7216-2	<i>brl1Δ::kanMX6 ade6-M210 ura4-D18 leu1-32 h⁺</i>	Bioneer V2
KGY7546-2	<i>bmt5Δ::kanMX6 ade6-M210 ura4-D18 leu1-32 h⁺</i>	BioneerV2

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References

- Bahler J, Wu JQ, Longtine MS, Shah NG, Mc Kenzie A, Steever AB, et al., Pringle JR. 1998. Heterologous modules for efficient and versatile PCR-based gene targeting in *Schizosaccharomyces pombe*. *Yeast*. 14: 943-51. 1579.
- Barbet N, Muriel WJ, Carr AM. 1992. Versatile shuttle vectors and genomic libraries for use with *Schizosaccharomyces pombe*. *Gene*. 114: 59-66. 789.
- Berry LD, Feoktistova A, Wright MD, Gould KL. 1999. The *Schizosaccharomyces pombe* dim1(+) gene interacts with the anaphase-promoting complex or cyclosome (APC/C) component lid1(+) and is required for APC/C function. *Mol Cell Biol*. 19: 2535-46. 1704.
- Berry LD, Gould KL. 1997. Fission yeast dim1(+) encodes a functionally conserved polypeptide essential for mitosis. *J Cell Biol*. 137: 1337-54. 3793. PubMed ID: [9182666](#)
- Bishop DT, Mc Donald WH, Gould KL, Forsburg SL. 2000. Isolation of an essential *Schizosaccharomyces pombe* gene, prp31(+), that links splicing and meiosis. *Nucleic Acids Res*. 28: 2214-20. 3518. PubMed ID: [10871341](#)
- Carme P, Rutherford K, Bahler J, Mata J, Wood V. 2026. PomBase in 2026: Expanding Knowledge, Modelling Connections. *Genetics* 3791. PubMed ID: [41518600](#)
- Carnahan RH, Feoktistova A, Ren L, Niessen S, Yates JR, Gould KL. 2005. Dim1p is required for efficient splicing and export of mRNA encoding lid1p, a component of the fission yeast anaphase-promoting complex. *Eukaryot Cell*. 4: 577-87. 2326. PubMed ID: [15755920](#)
- Cingolani P, Platts A, Wang Le L, Coon M, Nguyen T, Wang L, et al., Ruden DM. 2012. A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff: SNPs in the genome of *Drosophila melanogaster* strain w1118; iso-2; iso-3. *Fly (Austin)*. 6: 80-92. 3800. PubMed ID: [22728672](#)
- Dobbyn HC, Mc Ewan PA, Krause A, Novak Frazer L, Bella J, O Keefe RT. 2007. Analysis of pre-mRNA and pre-rRNA processing factor Snu13p structure and mutants. *Biochem Biophys Res Commun*. 360: 857-62. 3776. PubMed ID: [17631273](#)
- Dobbyn HC, O Keefe RT. 2004. Analysis of Snu13p mutations reveals differential interactions with the U4 snRNA and U3 snoRNA. *RNA*. 10: 308-20. 3777. PubMed ID: [14730029](#)
- Fair BJ, Pleiss JA. 2017. The power of fission: yeast as a tool for understanding complex splicing. *Curr Genet*. 63: 375-380. 3706. PubMed ID: [27628706](#)
- Forsburg SL, Rhind N. 2006. Basic methods for fission yeast. *Yeast*. 23: 173-83. 3291. PubMed ID: [16498704](#)
- Gibson DG, Young L, Chuang RY, Venter JC, Hutchison CA, Smith HO. 2009. Enzymatic assembly of DNA molecules up to several hundred kilobases. *Nat Methods*. 6: 343-5. 3611. PubMed ID: [19363495](#)
- Gottschalk A, Neubauer G, Banroques J, Mann M, Luhrmann R, Fabrizio P. 1999. Identification by mass spectrometry and functional analysis of novel proteins of the yeast [U4/U6.U5] tri-snRNP [In Process Citation]. *Embo J*. 18: 4535-48. 1708.
- Herissant L, Moehle EA, Bertaccini D, Van Dorsselaer A, Schaeffer Reiss C, Guthrie C, Dargemont C. 2014. H2B ubiquitylation modulates spliceosome assembly and function in budding yeast. *Biol Cell*. 106: 126-38. 3785. PubMed ID: [24476359](#)
- Keeney JB, Boeke JD. 1994. Efficient targeted integration at leu1-32 and ura4-294 in *Schizosaccharomyces pombe*. *Genetics*. 136: 849-56. 806.
- Li H. 2018. Minimap2: pairwise alignment for nucleotide sequences. *Bioinformatics*. 34: 3094-3100. 3797. PubMed ID: [29750242](#)
- Lock A, Rutherford K, Harris MA, Wood V. 2018. PomBase: The Scientific Resource for Fission Yeast. *Methods Mol Biol*. 1757: 49-68. 3426. PubMed ID: [29761456](#)
- Moehle EA, Ryan CJ, Krogan NJ, Kress TL, Guthrie C. 2012. The yeast SR-like protein Npl3 links chromatin modification to mRNA processing. *PLoS Genet*. 8: e1003101. 3784. PubMed ID: [23209445](#)
- Moreno S, Klar A, Nurse P. 1991. Molecular genetic analysis of fission yeast *Schizosaccharomyces pombe*. *Methods Enzymol*. 194: 795-823. 532. DOI: [10.1016/0076-6879\(91\)94059-1](#)
- Nonaka N, Kitajima T, Yokobayashi S, Xiao G, Yamamoto M, Grewal SI, Watanabe Y. 2002. Recruitment of cohesin to heterochromatic regions by Swi6/HP1 in fission yeast. *Nat Cell Biol*. 4: 89-93. 3781. PubMed ID: [11780129](#)

Potashkin J, Kim D, Fons M, Humphrey T, Frendewey D. 1998. Cell-division-cycle defects associated with fission yeast pre-mRNA splicing mutants. *Curr Genet.* 34: 153-63. 1642.

Potashkin J, Li R, Frendewey D. 1989. Pre-mRNA splicing mutants of *Schizosaccharomyces pombe*. *Embo J.* 8: 551-9. 1672.

Reuter K, Nottrott S, Fabrizio P, Luhrmann R, Ficner R. 1999. Identification, characterization and crystal structure analysis of the human spliceosomal U5 snRNP-specific 15 kD protein. *J Mol Biol.* 294: 515-25. 2168. PubMed ID: [10610776](#)

Rutherford KM, Lera Ramirez M, Wood V. 2024. PomBase: a Global Core Biodata Resource-growth, collaboration, and sustainability. *Genetics* 3579. PubMed ID: [38376816](#)

Schindelin J, Arganda Carreras I, Frise E, Kaynig V, Longair M, Pietzsch T, et al., Cardona A. 2012. Fiji: an open-source platform for biological-image analysis. *Nat Methods.* 9: 676-82. 3292. PubMed ID: [22743772](#)

Stevens SW, Abelson J. 1999. Purification of the yeast U4/U6.U5 small nuclear ribonucleoprotein particle and identification of its proteins. *Proc Natl Acad Sci U S A.* 96: 7226-31. 1709.

Tanny JC, Erdjument Bromage H, Tempst P, Allis CD. 2007. Ubiquitylation of histone H2B controls RNA polymerase II transcription elongation independently of histone H3 methylation. *Genes Dev.* 21: 835-47. 3783. PubMed ID: [17374714](#)

Urushiyama S, Tani T, Ohshima Y. 1996. Isolation of novel pre-mRNA splicing mutants of *Schizosaccharomyces pombe*. *Mol Gen Genet.* 253: 118-27. 1673.

Wilkinson ME, Charenton C, Nagai K. 2020. RNA Splicing by the Spliceosome. *Annu Rev Biochem.* 89: 359-388. 3792. PubMed ID: [31794245](#)

Willet AH, Ren L, Turner LA, Gould KL. 2023. Isolation of mutant alleles of the U6 snRNA m(6) A methyltransferase Mtl16 and characterization of their genetic interactions with splicing mutants in *Schizosaccharomyces pombe*. *MicroPubl Biol.* 2023 3703. PubMed ID: [37637271](#)

Wood V, Harris MA, Mc Dowall MD, Rutherford K, Vaughan BW, Staines DM, et al., Oliver SG. 2012. PomBase: a comprehensive online resource for fission yeast. *Nucleic acids research.* 40: D695-9. 3024. PubMed ID: [22039153](#)

Yun T, Li H, Chang PC, Lin MF, Carroll A, Mc Lean CY. 2021. Accurate, scalable cohort variant calls using DeepVariant and GLnexus. *Bioinformatics.* 36: 5582-5589. 3799. PubMed ID: [33399819](#)

Zhang Y, Lindblom T, Chang A, Sudol M, Sluder AE, Golemis EA. 2000. Evidence that dim1 associates with proteins involved in pre-mRNA splicing, and delineation of residues essential for dim1 interactions with hnRNP F and Npw38/PQBP-1. *Gene.* 257: 33-43. 1994. PubMed ID: [11054566](#)

Zhang YZ, Gould KL, Dunbrack RJ, Cheng H, Roder H, Golemis EA. 1999. The evolutionarily conserved Dim1 protein defines a novel branch of the thioredoxin fold superfamily. *Physiol Genomics.* 1: 109-18. 3514. PubMed ID: [11015569](#)

Zheng Z, Li S, Su J, Leung AW, Lam TW, Luo R. 2022. Symphonizing pileup and full-alignment for deep learning-based long-read variant calling. *Nat Comput Sci.* 2: 797-803. 3798. PubMed ID: [38177392](#)

Zofall M, Grewal SI. 2007. HULC, a histone H2B ubiquitinating complex, modulates heterochromatin independent of histone methylation in fission yeast. *J Biol Chem.* 282: 14065-72. 3782. PubMed ID: [17363370](#)

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