

## A

Ptr H3	1	MARTKQTARKSTGGKAPRKQLASKAARKSAPSTGGVKKPHRYKPGTVALREIRRYQKSTE
Sn H3	1	MARTKQTARKSTGGKAPRKQLASKAARKSAPSTGGVKKPHRYKPGTVALREIRRYQKSTE
NcH3	1	MARTKQTARKSTGGKAPRKQLASKAARKSAPSTGGVKKPHRYKPGTVALREIRRYQKSTE
ScH3	1	MARTKQTARKSTGGKAPRKQLASKAARKSAPSTGGVKKPHRYKPGTVALREIRRYQKSTE
SpH3	1	MARTKQTARKSTGGKAPRKQLASKAARKAAPATGGVKKPHRYRPGTVALREIRRYQKSTE
HsH3.1	1	MARTKQTARKSTGGKAPRKQLATKAARKSAPATGGVKKPHRYRPGTVALREIRRYQKSTE
HsH3.3	1	MARTKQTARKSTGGKAPRKQLATKAARKSAPSTGGVKKPHRYRPGTVALREIRRYQKSTE
SNOG_03319	61	LLIRKLPFQRLVREIAQDFKSDLRFQSSAIGALQESVEAYLVSLFEDTNLCAIHAKRVTI
PTRG_11558.1	61	LLIRKLPFQRLVREIAQDFKSDLRFQSSAIGALQESVEAYLVSLFEDTNLCAIHAKRVTI
NcH3	61	LLIRKLPFQRLVREIAQDFKSDLRFQSSAIGLLQESVESYLVSLFEDTNLCAIHAKRVTI
ScH3	61	LLIRKLPFQRLVREIAQDFKTLDRFQSSAIGALQESVEAYLVSLFEDTNLAAIHAKRVTI
SpH3	61	LLIRKLPFQRLVREIAQDFKTLDRFQSSAIGALQEAVEAYLVSLFEDTNLCAIHGKRVTI
HsH3.1	61	LLIRKLPFQRLVREIAQDFKTLDRFQSSAVMALQEAACEAYLVGLFEDTNLCAIHAKRVTI
HsH3.3	61	LLIRKLPFQRLVREIAQDFKTLDRFQSSAAIGALQEAASEAYLVGLFEDTNLCAIHAKRVTI
SNOG_03319	121	QSKDIQLARRLRGERG
PTRG_11558.1	121	QSKDIQLARRLRGERG
NcH3	121	QSKDIQLARRLRGERN
ScH3	121	QKKDIKLARRLRGERS
SpH3	121	QPKDMQLARRLRGERS
HsH3.1	121	MPKDIQLARRIRGERA
HsH3.3	121	MPKDIQLARRIRGERA

## B

SNOG_15370	1	MAPKTPDRRNKARPSRGGRAS-----TGSRRRESGKRVGQAGFAPDRAKKRYKPGTVAL
PtrCenH3	1	MAPKSPARRNKARPSRGGRVS-----VGSRKESGKRLGQPGFAPDRAKKRYKPGTVAL
Sc_Cse4	1	METEVP-----PVRTHSYA-----LDRYVRQKRREKQRKQSLKRVEKKYTPSELAL
Nc_CenH3	1	MPPKGGVTKSAVSKKAAAVPTPKATPPGRRKSRASSVQGDVFPQGGKRRYRPGTLAL
Sp_Cnp1	1	-----MAKKSLEMA-----EPGDFIPRPRKKRYRPGTTAL
MmCENpA	1	MGPFR---KPQTPRRR-PS-S-----PAPGFSRQSSSVGSQTLRR-RQKFMWL
HsCENpA	1	MGPFRRRSRKPEAPRRRSPSP-----PTPGFSRRGPSLGASSHQHSRRRQGWL
SNOG_15370	54	REIRRYQKSTDLLLRLTPFQRLVREIAQTVTT---ETGPTRWQSQAIQALQEAATEAFLVN
PtrCenH3	54	REIKRYQKTTDLLLKLFPFQRLVREIAQSVTT---EDGPNRWQSQAIMALQEAATEAFLVN
Sc_Cse4	48	YEIRKYQRSTDLLISKIPFARLVREIKVTFEFT---KDQDLRWQSMAIMALQEAASEAYLVG
Nc_CenH3	61	KEIRNYQRTTDLVAKLPPFARLVREIAMQFRP---MDEEMRWQSQAILALQEAEEAFLVH
Sp_Cnp1	30	REIRKYQRSTDLIIQRLPFSRIVREISSEFVANFSTDVGLRWQSTALQCLQEAEEAFLVH
MmCENpA	43	KEIKTLQKSTDLFRKKPFSMVREICEKFSR---GVDFNWQAQALLALQEAEEAFLIH
HsCENpA	49	KEIRKLQKSTHLLIRKLPFSRLAREICVKFTR---GVDFNWQAQALLALQEAEEAFLVH
SNOG_15370	111	LFHDANLCAIHAKRVTIQKDIQLARRLRAANGAPV--
PtrCenH3	111	LFHDANLCAIHAKRVTIQKDIQLARRLRAANGAPV--
Sc_Cse4	105	LEHTNLLALHAKRITIMKKDMQLARRIRGQFI----
Nc_CenH3	118	LFEDTNLCAIHAKRVTIMQKDIQLARRIRGVWGAGWV
Sp_Cnp1	90	LFEDTNLCAIHAKRVTIMQDMQLARRIRGA-----
MmCENpA	99	LFEDAYLLSLHAGRVTLFPPKDIQLTRRIRGFEGGLP--
HsCENpA	105	LFEDAYLLTLHAGRVTLFPPKDVQLARRIRGLEEGLG--

**Figure S4** Alignment of *bona fide* histone H3 proteins from *Ptr* with those of other fungi reveal the expected level of conservation. (A) Histone H3 is extremely well conserved among *Ptr*, *S. nodorum* (Sn), *N. crassa* (Nc), *Schizosaccharomyces pombe* (Sp), *Saccharomyces cerevisiae* (Sc) and strongly resembles the replication-dependent H3.1 or replication-independent H3.3 from human (Hs). This is in stark contrast to alignments with the novel family of H3L proteins identified in *Ptr* (see Figure 3B). (B) Centromere-specific variants of H3, called CenH3 or CenpA, have variable N-terminal regions and more conserved histone-fold and C-terminal domains. While this variability suggested that H3Ls may be similar to CenH3 proteins, alignments of the histone-fold domain suggest that H3Ls are derived from canonical H3s (see Figure 3B). In both figures, completely conserved residues are shown in black, similar residues are in green and variable residues are in red.