Supplemental Figure S1A

ura4 WT

ATGGATGCTAGAGTATTTCAAAGCTATTCAGCTAGAGCTGAGGGGATGAAAAATCCCATTGCCAAGGAATTGTTGGCTTTG
ATGGAAGAAAAGCAAAGCAACTTGTCAGTCGCGGTCGATTTGACGAAGAAATCCGAAATCTTAGAATTGGTAGATAAAATT
GGACCCTATGTCTGTGTTATCAAGACACATATTGACGTTGTCGAGGATTTCGACCAGGATATGGTAGAAAAACTGGTGGCC
TTAGGTAAAAAGCATCGTTTTCTTATCTTTGAGGATCGCAAATTCGCAGACATTGGAAATACCGTCAAGCTACAATATGCAT
CTGGTGTGTACAAAATTGCTTCTTGGGCTCATATCACAAATTGCCATACAGTGCCAGGCGAGGGTATTATACAAGGCCTCAA
AGAAGTTGGTTTACCTTTGGGACGTGGTCTCTTGCTTTTGGCTGAAATGTCTTCCAAAGGCTCTTTGGCTACTGGTTCCTAC
ACAGAGAAAACCTTAGAATGGTTTGAGAAGCATACCGATTTTTGCTTTGGCTTTATAGCTGGTCGTCGATTTCCTAACCTTCA
AAGCGACTACATAACTATGTCCCCTGGTATCGGCTTGGATGTTAAAGGAGACGGGCTGGGACAGCAATATCGTACTCCTGA
AGAAGTGATTGTAAACTGCGGTAGCGATATCATCATTGTTGGTCGTGGAGTCTATGGAGCTGGTCGTAATCCTGTTGTCGA
AGCCAAGAGATATAGAGAAGCTGGTTGGAAGGCATATCAGCAAAGACTTTCTCAGCATTAA

ura4 1_6

ATGGACGCGCGCGTGTTCCAGTCATACAGCGCGCGCGCGGAAGGTATGAAGAACCCGATCGCTAAAGAGCTACTAGCGCT
AATGGAGGAGAAACAGTCAAATCTAAGCGTAGCTGTAGACCTAACTAAAAAGAGTGAGATACTCGAGCTAGTCGACAAGAT
CGGGCCATACGTATGCGTCATTAAAACGCACATCGATGTCGTAGAAGACTTTGATCAAGACATGGTCGAGAAGTTAGTAGC
ACTCGGCAAGAAACACAGGTTCTTGATATTCGAAGACAGAAAGTTTGCGGATATCGGCAACACTGTTAAATTGCAGTACGC
GAGCGGCGTATATAAGATCGCGAGCTGGGCGCACATAACGAACTGTCACACCGTACCGGGTGAAGGCATCATCCAGGGAT
TAAAGGAGGTCGGCCTGCCGCTAGGCAGGGGCTTACTATTGCTAGCAGAGATGAGCAGTAAGGGAAGCCTAGCGACGGG
CAGTTATACGGAAAAGACACTCGAGTGGTTCGAAAAACACACTGACTTCTGTTTCGGATTCATCGCGGGCAGGAGGTTCCC
GAATTTGCAGTCAGATTATATCACGATGAGTCCGGGCATAGGACTAGACGTCAAGGGCGATGGATTAGGCCAACAGTACAG
GACGCCGGAGGAGGTAATCGTCAATTGTGGCTCAGACATTATTATCGTCGGCAGGGGCGTATACGGCGCCGGCAGGAACC
CGGTCGTAGAGGCTAAACGCTACCGCGAGGCCGGCTGGAAAGCGTACCAACAGCGCTTGAGCCAACACTAA

ura4 1_3

ATGGACGCGCGCGTGTTCCAGTCATACAGCGCGCGCGCGGAAGGTATGAAGAACCCGATCGCTAAAGAGCTACTAGCGCT
AATGGAGGAGAAACAGTCAAATCTAAGCGTAGCTGTAGACCTAACTAAAAAGAGTGAGATACTCGAGCTAGTCGACAAGAT
CGGGCCATACGTATGCGTCATTAAAACGCACATCGATGTCGTAGAAGACTTTGATCAAGACATGGTCGAGAAGTTAGTAGC
ACTCGGCAAGAAACACAGGTTCTTGATATTCGAAGACAGAAAGTTTGCGGATATCGGCAACACTGTTAAATTGCAGTACGC
GAGCGGCGTATATAAGATCGCGAGCTGGGCGCACATAACGAACTGTCACACCGTACCGGGTGAAGGCATCATACAAGGCC
TCAAAGAAGTTGGTTTACCTTTGGGACGTGGTCTCTTGCTTTTGGCTGAAATGTCTTCCAAAGGCTCTTTGGCTACTGGTTC
CTACACAGAGAAAACCTTAGAATGGTTTGAGAAGCATACCGATTTTTGCTTTGGCTTTATAGCTGGTCGTCGATTTCCTAAC
CTTCAAAGCGACTACATAACTATGTCCCCTGGTATCGGCTTGGATGTTAAAGGAGACGGGCTGGGACAGCAATATCGTACT
CCTGAAGAAGTGATTGTAAACTGCGGTAGCGATATCATCATTGTTGGTCGTGGAGTCTATGGAGCTGGTCGTAATCCTGTT
GTCGAAGCCAAGAGATATAGAGAAGCTGGTTGGAAGGCATATCAGCAAAGACTTTCTCAGCATTAA

ura4 Shuffled

ATGGACGCGCGTGTCTTCCAGTCCTACTCTGCCCGAGCAGAAGGCATGAAGAACCCAATCGCCAAAGAGCTCCTGGCCCT
AATGGAGGAAAAGCAAAGCAATCTCTCAGTGGCTGTGGACCTGACGAAGAAGTCAGAAATCTTAGAGCTTGTTGACAAAAT
TGGCCCCTACGTTTGTGTTATTAAGACCCATATAGACGTCGTGGAAGACTTTGATCAAGATATGGTAGAAAAACTTGTTGCA
TTGGGTAAGAAACATCGTTTCCTTATCTTTGAGGATCGTAAATTTGCTGATATCGGAAACACTGTGAAATTACAGTACGCAA
GCGGCGTTTATAAAATTGCTAGCTGGGCTCATATTACCAACTGCCATACCGTCCCTGGAGAAGGGATAATCCAGGGACTTA
AGGAAGTAGGATTACCTTTAGGAAGAGGGTTGTTGTTGTTGGCTGAAATGTCCAGCAAAGGATCCTTGGCTACTGGTTCCT
ATACTGAAAAAACATTGGAATGGTTTGAGAAGCATACAGATTTTTGCTTTGGCTTTATTGCTGGAAGACGCTTTCCCAATTTG
CAGTCTGATTATATAACAATGTCTCCTGGCATTGGTTTGGATGTAAAGGGTGATGGTTTGGGTCAACAATATCGTACACCTG
AGGAAGTTATCGTAAATTGCGGTTCTGATATTATCATTGTCGGTAGAGGTGTCTATGGTGCTGGTAGAAATCCTGTCGTCGA
AGCTAAACGTTATAGAGAAGCTGGTTGGAAAGCTTATCAACAACGTTTGTCTCAACATTAA

Supplemental Figure S1B

Nucleosomes 3-4
Chromosome I: Positions 2753585 to 2753879 (Intergenic region SPAC6F6.11c-SPAC6F6.12)

Wild type sequence
GATAATCAACTTAATTTTTTGCGACAAAACTAATTATGAAGCACATTCTTTTGAATCAATTTATCCAAAAAAGTAATAAATTCA
ACAGTGACAAGATGTAAGCATTATACGGAATACAACGACTCGAAATACATGGAATTGTATAACTTAATCACTCATTTAAACTT
TTACAGAAATCTTCGGCTGAAATATACGGGGTAAAATAGGGCTTTTAATTGTAAAGCTTAGAGCCTGAATAAGTATGCTTTC
CTTTATTTTAACATTCCTCAATTTGTTTTCAAGTACTTCACATACC

Randomized sequence
GGTACTAATATCAACATTTAAAATGTGATTTGGTTCTTTATCTTATGTAACCTTAGAAATAATTTTTTATACGACAAATAACTT
GTGGCCTTGTAACATCGGTATAAACAATTTCATTAATATTAAATATTAATTATACAGAGTACAGTTCAAATCTAAAGTAGTTC
GCAACTCTACTCTCGCATCCTCTCGATAAACAAACATAGAAGTAATACAAAAGCCAGAAATTGCTCTAAAAATGAATTTTGTA
CCGTAGGTATCCTTTGTGTTACCGCATATAAATACTGATACGAAG

Supplemental Figure S1C

Nucleosome 1
Chromosome III: Positions 2236704 to 2236793 (reverse complementary)

TATAGTATCAAATTTTTGTGTCTTAAGCTTTTTATGCAGATGCAGTTTCGTTGCATCAATTTCATGCATAACCTTTCCCCCATT
TATTTC

Nucleosome 2
Chromosome III: Positions 844797 to 844949

GATACAAGGACTCTAAAAATCGCCAGATACAGAATGCGTCAAAACCTTGAGAAGGAGTTAAAGGCTTTGTCTAAGGACTCG
GAAACCAATGAAGAGCAGGAGAGGAAATTTTGGCTCACTAAATTACAAATTGCTGTTGAAGATACATTGGAC

Nucleosome 3
Chromosome II: Position 3119782 to 3119934 (reverse complementary)
GTTTTTCAATACTAGGAGATCGCTATCCAGAATGCAGACCTTATCAAACTGTGTTTGCTCAAATACACTGAGTTTTGTGAACA
TGTATTGATAACGATAACTGCCCATGCCAATAGACAAACCACTTTCAATTAAGTCTTCAGTTTTAATTTG

Nucleosome 4
Chromosome I: Position 2522247 to 2522399
TATTTTTCTCAAGGCGATTTTTCGAACTTAGATGTACTACGAGATTTTTTTTCATGTACTCACTAGGAAGGAGCAAGATGTCCA
CATTGCTCTTAACATCAACTTGAAAAGTTTTTTGTGCGAATGGAGACAGAATGCTCTTGTTCTACTAAAA

Nucleosome 5
Chromosome I: Position 1502152 to1502304
AATACCAATAAGTCACACCAAAGGTCTTTTTCAATTCCAAAAGCAACGAAGGATTCTCAAACTCCGTCGGAAAATAGTGCTG
CAACTTTAAAACAAGCTGCAATTGATGCGTATTCTCAAATTCCTGTCATTCCTTTTTTTCTTCCCAGTGAT

Nucleosome 6
Chromosome I: Position 807303 to 807389
GTTCCATCTACTAGAACATTTAGTCAGCTTCTAAACACAAAGCAGGCTTCTTTCCTTGATTTTCTTTCAAAATGTTTGAAATG
GGAT

Chimaeric sequence
GAAATAAATGGGGGAAAGGTTATGCATGAAATTGATGCAACGAAACTGCATCTGCATAAAAAGCTTAAGACACAAAAATTTG
ATACTATAGATACAAGGACTCTAAAAATCGCCAGATACAGAATGCGTCAAAACCTTGAGAAGGAGTTAAAGGCTTTGTCTAA
GGACTCGGAAACCAATGAAGAGCAGGAGAGGAAATTTTGGCTCACTAAATTACAAATTGCTGTTGAAGATACATTGGACCA
AATTAAAACTGAAGACTTAATTGAAAGTGGTTTGTCTATTGGCATGGGCAGTTATCGTTATCAATACATGTTCACAAAACTCA
GTGTATTTGAGCAAACACAGTTTGATAAGGTCTGCATTCTGGATAGCGATCTCCTAGTATTGAAAAACTATTTTTCTCAAGGC
GATTTTTCGAACTTAGATGTACTACGAGATTTTTTTTCATGTACTCACTAGGAAGGAGCAAGATGTCCACATTGCTCTTAACAT
CAACTTGAAAAGTTTTTTGTGCGAATGGAGACAGAATGCTCTTGTTCTACTAAAAAATACCAATAAGTCACACCAAAGGTCTT
TTTCAATTCCAAAAGCAACGAAGGATTCTCAAACTCCGTCGGAAAATAGTGCTGCAACTTTAAAACAAGCTGCAATTGATGC
GTATTCTCAAATTCCTGTCATTCCTTTTTTTCTTCCCAGTGATGTTCCATCTACTAGAACATTTAGTCAGCTTCTAAACACAAA
GCAGGCTTCTTTCCTTGATTTTCTTTCAAAATGTTTGAAATGGGAT

Randomized sequence
ACTAGCCATGGAGACCGAAATGTAAACGTGAAGGAGGCAATATATTATCATGGAGAAATCTATTTTAAAAAGAAATGATGAA
TGAAACTTAACTGAGACATAACATGGTGAGTATCAATTCAAAAACTATGCGGTATAAAAACATTATGTCAAGCTACATCAGA
GGGATCGACGGCACACTTAGAAGAATTAGTGCTGACATCTCGATGAAACTCGTGTAAAGGGAGCCAGAAGCATGAGAATT
GATAGTAATACCGCCGACAGTTTTTTATAAAAAATGGAGTTAGTTACCGGACCATATTCATTTAAATGTTATGTATAGAATCG
CGTCATTGAACCTATTGCAAAAGAGCATCGGATGTTAGTGTCGACTGATGAATCTCGGAGTAAATACACTTACGGTTCCTTA
TAAAAAGGATCATTATTACGGTCTAATCTATGAAAGTGTTTGCTTAAGAGTTTTGCGTGATTTGACTCCCTGTATCTTGAAAA
ATACAGAGTTTGGTCAAGGATTCTAGTAGTATACTCTTCAATCAATGACTACCTGACCATACTAAATCATCGAAATCATCATT
TCACTCCAATACTATCAGTATATAACACGCCAGGGACCTCTTACCCAATTGCCATTAATTGATCAGGCCAAAGGACGTTAAT
GATACTCCTTTGAATCATAATTGGACTCATTGAACAGGTTTTGTTCCTCACCAAAGTTTAATGCGATGATAAATGATATACTC
ATATATTATTAGTTCTGTAATGTATTGCTTGTTCTTTTCGACAACCCCGCAACGT

Supplemental Figure S1D

ura4 dyad

ATGGACGCGCGCGTGTTCCAGTCATACAGCGCGCGCGCGGAAGGGATGAAAAATCCCATTGCCAAGGAATTGTTGGCTTT
GATGGAAGAAAAGCAAAGCAACTTGTCAGTCGCGGTCGATTTGACGAAGAAATCCGAAATACTCGAGCTAGTCGACAAGAT
CGGGCCATACGTATGCGTCATTAAAACGCATATTGACGTTGTCGAGGATTTCGACCAGGATATGGTAGAAAAACTGGTGGC
CTTAGGTAAAAAGCATCGTTTTCTTATCTTTGAGGATCGCAAATTCGCAGACATTGGAAATACCGTTAAATTGCAGTACGCG
AGCGGCGTATATAAGATCGCGAGCTGGGCGCACATCACAAATTGCCATACAGTGCCAGGCGAGGGTATTATACAAGGCCT
CAAAGAAGTTGGTTTACCTTTGGGACGTGGTCTCTTGCTTCTAGCAGAGATGAGCAGTAAGGGAAGCCTAGCGACGGGCA
GTTATACGGAAAAAACCTTAGAATGGTTTGAGAAGCATACCGATTTTTGCTTTGGCTTTATAGCTGGTCGTCGATTTCCTAAC
CTTCAAAGCGACTACATAACTATGTCCCCTGGTATCGGACTAGACGTCAAGGGCGATGGATTAGGCCAACAGTACAGGACG
CCGGAGGAAGTGATTGTAAACTGCGGTAGCGATATCATCATTGTTGGTCGTGGAGTCTATGGAGCTGGTCGTAATCCTGTT
GTCGAAGCCAAGAGATATAGAGAAGCTGGTTGGAAGGCATATCAGCAAAGATTGAGCCAACACTAA

ura4 linker

ATGGATGCTAGAGTATTTCAAAGCTATTCAGCTAGAGCTGAGGGGATGAAAAATCCCATTGCCAAGGAATTGTTGGCGCTA
ATGGAGGAGAAACAGTCAAATCTAAGCGTAGCTGTAGACCTAACGAAGAAATCCGAAATCTTAGAATTGGTAGATAAAATT
GGACCCTATGTCTGTGTTATCAAGACACATATTGACGTTGTCGAGGATTTCGACCAGGATATGGTAGAGAAGTTAGTAGCA
CTCGGCAAGAAACACAGGTTCTTGATATTCGAAGATCGCAAATTCGCAGACATTGGAAATACCGTCAAGCTACAATATGCAT
CTGGTGTGTACAAAATTGCTTCTTGGGCTCATATCACAAATTGCCATACAGTACCGGGTGAAGGCATCATCCAGGGATTAAA
GGAGGTCGGCCTGCCGTTGGGACGTGGTCTCTTGCTTTTGGCTGAAATGTCTTCCAAAGGCTCTTTGGCTACTGGTTCCTA
CACAGAGAAAACCTTAGAATGGTTTGAGAAGCATACCGATTTTTGCTTTGGCTTTATCGCGGGCAGGAGGTTCCCGAATTT
GCAGTCAGATTATATCACGATGAGCCCTGGTATCGGCTTGGATGTTAAAGGAGACGGGCTGGGACAGCAATATCGTACTCC
TGAAGAAGTGATTGTAAACTGCGGTAGCGATATCATCATTGTTGGTCGTGGAGTCTATGGAGCCGGCAGGAACCCGGTCGT
AGAGGCTAAACGCTACCGCGAGGCCGGCTGGAAGGCATATCAGCAAAGACTTTCTCAGCATTAA

ura4 int

ATGGATGCTAGAGTATTTCAAAGCTATTCAGCTAGAGCTGAGGGGATGAAGAACCCGATCGCTAAAGAGCTATTGGCTTTG
ATGGAAGAAAAGCAAAGCAACTTGTCAGTCGCGGTCGATCTAACTAAAAAGAGTGAGATACTCGAATTGGTAGATAAAATT
GGACCCTATGTCTGTGTTATCAAGACACATATTGACGTCGTAGAAGACTTTGATCAAGACATGGTAGAAAAACTGGTGGCCT
TAGGTAAAAAGCATCGTTTTCTTATCTTTGAGGATAGAAAGTTTGCGGATATCGGCAACACCGTCAAGCTACAATATGCATC
TGGTGTGTACAAAATTGCTTCTTGGGCTCACATAACGAACTGTCACACCGTACCAGGCGAGGGTATTATACAAGGCCTCAA
AGAAGTTGGTTTACCTCTAGGCAGGGGCTTACTATTGCTAGCTGAAATGTCTTCCAAAGGCTCTTTGGCTACTGGTTCCTAC
ACAGAGAAAACCTTAGAATGGTTCGAAAAACACACTGACTTCTGCTTTGGCTTTATAGCTGGTCGTCGATTTCCTAACCTTC
AAAGCGACTACATAACTATGAGTCCGGGCATAGGACTAGACGTTAAAGGAGACGGGCTGGGACAGCAATATCGTACTCCT
GAAGAAGTGATTGTAAACTGTGGCTCAGACATTATTATCGTCGGTCGTGGAGTCTATGGAGCTGGTCGTAATCCTGTTGTC
GAAGCCAAGAGATATAGAGAAGCTGGCTGGAAAGCGTACCAACAGCGCCTTTCTCAGCATTAA

Supplemental Figure S1E

S. pombe

CCAGGAAGAATGAGAGCAGGACTCTTCCTCTGAAACCTCGACGGAATTCCAGCAGAAAGTAAATGTAGTTTAAGAATTAGA
CCTAGACTTCCTCTCACTTACTCTTGGGCTTGAGGTGAGTGGGATGCTGGTGGTGATACTCCTGATGATGAACAATCAGCA
GCATAAGGAGTCAGAAACAGCGCATGGGGCACAAACGCAAGCGCAATAGCAAGAGGACCAGCTAAATATTGTAGAAGAGT
TAGATGTGGTCTTCCTCTGCACAATTCGGACAAGACTTTGGGTACTGTTCGTGGTAGGGGTCCCAGATAAGCACCATGGAA
AAGAACAGGAACAAACGCATGAAGAAGCTGACTTTAACATTTAAGTGTACAAGGAGTAAGACCTTTATATCCTGGTCTTGAA
GGCTGAAGGGACATCTGGCAGACTGCGACTGCTTGTAAGGCTCGTCCTCCTCGTACAAGACTAACAAGAGGACTCAGACA
CATCAGCAGATGCCAGCCACACGGATTATGTGGAACATCAAGAAGAGGATTAGGTTTACCTGAAGAACAAGTTCTTCGTCT
CTCTACTCTTCATGCTCCTGAGGGTGTTCATATTAGAGCTGCTCCCGGTAGAAGACCAAGAGAAGAACCAGGAAAATGAAG
AGCAAGCGTCGCATTCAGCATATAAGTAGTAGTATGGAGATGTAGATTACAATTAATTTTTCATTCTGTCTCTGTTGGTCGTT
CTGGTGCTTTGTCTCATTTTGGTGCTTTTTTAGCTTTTGGACTAGGACCAACATGCTCACACGCAGTCAGACTCGCAAGAGC
ACCAGAAGGAGGATTAATGATGAGAAAATTACCTGGTATTGTTTGTATCTGTGGGAATCGCTTTCTTGAGCGTTTTCGTGAT
CGTTGTAATTCTGGTTTTTTTTAC

S. cerevisiae

TATAGTTTCTGACGATACCTAAGAAAAAAACTTACCTAAGCACGCCAAGCCCCCGCGCGAGGCCCTGGGGGCCCACGGAA
CGGCATGGGCTGGTGCCAGCCGCCACATCCGTCCAAGGGTTAGCGGCTTTCCTTTTTTGTATGTACTATAAGATATTTATTC
AACGTCTAAGTGATAAAATATGTAAAAAAGATGCAACAACGAGCACTCCATCGGGCTGACGCGAGCGGTGCTTTCCAGCAC
ACCAGGTTGGTGTGAACCGTCAAGGTGCGACGACCTCCCCGGGCTTTGCCGCATTATATGTGTTTAGATAAACGTATTTAA
GGATATCTTCTACATGAATATATAGCTTCTAGAAAAAAAAAACGTGAACGCATCGTGGCGTCGCTACGCAGGGCATCAGTTC
GAGGAGCGAGCAGGCCGCGTAAAGTGCGCCCGTCGCTCCGGGTGCGTCGTTGCGTGTGTTAGATTTTCATTATATGAAATT
GTTATCTCTCACGATACGCAGGTTATATTAAAAAGCTCAAAGGAGATCCTAAAGCGCCCCAGGTCCAACCACACAGGAGCA
CACTCACCAGTCCGAGGGTTTGCGTGGCCGAGCCAACGGTGGGTGCACGCGGCCACCGTCGTCTGCTTTGATTTTACAAC
AATTATGAGACATGTTAATTAATTTGATAATAAGAATTCTATGAAAGGACGACGCAATGACGCGCCCTACAGCCCACCCTCC
CCCCTCCGATGGGAGGTGGCATGTTGTGAGACGTCGCCCCCTGGCGAGATGTTGAGGTACTTTGCCTCAGCTATTACATAT
CTTATTTGTTATTATATTGTGGAAATGACGAGGAAATAATGCAAAGCGAAATAAAAAGGCAATACAGACAAGACCGCCACCT
CCGCGTTCGAGTACAACTCCATCGAGTCCACCACACCCTGGCTGTGAGCTGGTCTCGGACGGCTAGGATTTTTTTTTATCTT
TCTATAATATTAATAACTAGACGTATTAACTCGC

Supplemental Figure S1F

WT *ura4* ORF *S. octosporus*

ATGGATGCTAGACTCAATTACCAATATTCACAAAGAGCAGATGGGTTAAGTAACCCTCTAGCAAAGGCTTTGCTTGCTTTAA
TGGAGCAAAAGCAATCGAATTTGTCTGTTGCCGTCGACTTGACAAAAAAAGCTGACATCCTTGCTCTCATCGAAAAAATTGG
TCCTTTTGTTTGTGTCATTAAGACGCATGTCGATGTCATTGAAGATTTTGATCAAGACTTTGTTCAGCAATTGACCGCCCTTT
CCGAGAAGCATCGATTCTTCATCTTTGAGGACCGTAAGTTTGCCGATATTGGAAACACCGTCAAGCTCCAATACTCTTCTGG
TGTTTACAAAATTGCTTCTTGGGCTCACATTACTAATTGTCATACTGTCCCTGGAGAAGGCATTGTTCAGGGTTTGAAAGAA
GTTGGTTTACCTCTTGGTCGCGGCTTGCTTCTCTTGGCGGAAATGTCTTCTAAAGGAAGTCTGGCCACTGGTTCTTACACAG
AAACTACTTTAGAATGGGCTGAAAAACATAGCGACTTTTGCATTGGCTTCATTGCCGGCCGTCGTTTCCCCAATCTTCAGCA
TGACTTTATCACTATGTCTCCCGGTATTGGATTGGATGTTAAAGGCGACGGTATGGGCCAGCAATACCGTACACCTCATGAA
GTTATTGTGAATTGTGGCAGCGATATTATCATCGTTGGCCGTGGAATCTATGGTGCTGGCCGGGATCCCGTAGTTGAAGCC
CAACGTTACAAAAAAGCAGGCTGGGATGCCTACCAAGAACGTCTTACCAAAAAATAA

WT *ura4* ORF *S. japonicus*

ATGTCGGACATCGCCTTGAAAACGTACACGGAGCGCGCCAATGTGCATCCTAATGCAGTCGCCAAGAAGTTGCTGCGTTTG
ATGGACGAGAAGAAGTCAAACCTCTCGGTCGCTGTGGACTTGACGAAGAAGAACCAAGTCCTGGAACTCGTTGACAAGATT
GGTCCCTCGATCTGCTTGTTGAAGACTCACATTGACATTGTGGAGGACTTTGACGCAGACATGGTCCAACAGCTGGTTGCT
CTGGCTGAGAAGCACAAGTTTTTGATTTTTGAGGATCGCAAGTTTGCCGACATTGGCAACACTGTCAAGCTTCAGTACTCTG
CTGGTGTGTACAAGATTGCTTCTTGGGCTGACATCACCAACTGCCACACTGTTCCCGGTGAGGGTATTATTAGCGGTTTGA
AGGAAGTTGGTCTTCCCTTGGGTCGTGGTTTGTTGCTTTTGGCTGAGATGTCTTCGAAGGGAACCCTGGCCACTGGAAGCT
ACACACAAGCCACATTGGAATTGGCTGAGAAGCACAACGACTTCTGTATGGGTTTTATCGCCAGACGCCGCTTCCCCGGTC
TCAAGAGCGACTTTATTCACATGACACCCGGTGTCGGTTTGGACGTTAAGGGCGATGGCCTTGGTCAACAATACCGTACAC
CAGAGGAAGTGATCTGTGAGAGCCAGAGCGATATCATTATCGTCGGTCGCGGTGTCTACGGCAGCGGCCGTGATGCTGCT
CAAGAAGCTGAGCGCTACAGAAAGGCCGGCTGGGAGGCCTACCAGCGTCGCATTTCCAAGCAGTAA

Modified *ura4* ORF *S. octosporus*

ATGGATGCTAGATTAAATTATCAATATTCTCAAAGAGCTGATGGCCTCTCGAATCCTCTTGCTAAGGCTCTTCTTGCTCTTAT
GGAACAGAAGCAATCAAATCTATCAGTAGCAGTAGACCTCACAAAGAAAGCAGACATCCTAGCTCTCATAGAAAAAATAGG
ACCTTTTGTTTGTGTTATTAAAACTCATGTTGATGTGATTGAGGATTTTGATCAGGATTTTGTTCAACAATTGACTGCTCTTTC
AGAAAAACATAGATTCTTCATATTCGAAGACAGAAAATTCGCAGACATAGGAAACACAGTAAAACTTCAATATTCTTCTGGA
GTTTATAAAATTGCTTCTTGGGCTCACATCACGAATTGTCATACTGTTCCTGGTGAGGGTATTGTACAGGGACTAAAAGAAG
TAGGATTACCACTAGGAAGAGGACTCCTCCTCCTCGCAGAGATGAGCTCAAAAGGATCTTTAGCTACTGGTTCTTATACTGA
GACTACTCTTGAGTGGGCTGAGAAGCATTCTGATTTTTGTATTGGTTTTATTGCTGGCAGAAGATTCCCAAATCTACAACAT
GACTTCATCACAATGAGCCCAGGAATAGGACTAGATGTAAAAGGAGATGGAATGGGTCAACAATATAGAACTCCTCATGAG
GTGATTGTGAATTGTGGTTCTGATATTATTATTGTTGGTCGTGGTATCTATGGAGCAGGAAGAGATCCAGTAGTAGAAGCAC
AGAGATACAAGAAAGCAGGCTGGGATGCTTATCAAGAAAGATTAACTAAAAAATAA

Modified *ura4* ORF *S. japonicus*

ATGAGTGATATAGCTTTAAAAACTTATACTGAAAGAGCTAATGTGCATCCGAATGCTGTTGCTAAGAAACTTCTTCGTCTTAT
GGATGAGAAGAAATCAAATCTATCAGTAGCAGTAGACCTCACAAAGAAGAACCAAGTCCTAGAGCTAGTAGATAAAATAGG
ACCTTCTATTTGTCTTTTAAAAACTCATATTGACATTGTTGAGGATTTTGATGCTGATATGGTTCAACAATTGGTTGCTCTGG
CAGAAAAACATAAATTCCTAATATTCGAAGACAGAAAATTCGCAGACATAGGAAACACAGTAAAACTTCAATATTCTGCTGG
AGTTTATAAAATTGCTTCTTGGGCTGACATCACGAATTGTCATACTGTTCCTGGTGAGGGTATTATAAGCGGACTAAAAGAA
GTAGGATTACCACTAGGAAGAGGACTCCTCCTCCTCGCAGAGATGAGCTCAAAAGGAACTTTAGCTACTGGTTCTTATACTC
AAGCTACTCTTGAGCTTGCTGAGAAGCATAATGATTTTTGTATGGGTTTTATTGCTAGAAGAAGATTCCCAGGATTAAAATC
AGACTTCATCCACATGACGCCAGGAGTAGGACTAGATGTAAAAGGAGATGGATTAGGTCAACAATATAGAACTCCTGAGGA
GGTGATCTGTGAGTCTCAGTCTGATATTATTATTGTTGGTCGTGGTGTCTATGGATCAGGAAGAGATGCAGCACAAGAAGC
AGAGAGATACAGAAAAGCAGGCTGGGAAGCTTATCAAAGAAGAATTTCTAAACAATAA

Supplemental Figure S1G

Kanamycin ORF (Wild type)

ATGGGTAAGGAAAAGACTCACGTTTCGAGGCCGCGATTAAATTCCAACATGGATGCTGATTTATATGGGTATAAATGGGCT
CGCGATAATGTCGGGCAATCAGGTGCGACAATCTATCGATTGTATGGGAAGCCCGATGCGCCAGAGTTGTTTCTGAAACAT
GGCAAAGGTAGCGTTGCCAATGATGTTACAGATGAGATGGTCAGACTAAACTGGCTGACGGAATTTATGCCTCTTCCGACC
ATCAAGCATTTTATCCGTACTCCTGATGATGCATGGTTACTCACCACTGCGATCCCCGGCAAAACAGCATTCCAGGTATTAG
AAGAATATCCTGATTCAGGTGAAAATATTGTTGATGCGCTGGCAGTGTTCCTGCGCCGGTTGCATTCGATTCCTGTTTGTAA
TTGTCCTTTTAACAGCGATCGCGTATTTCGTCTCGCTCAGGCGCAATCACGAATGAATAACGGTTTGGTTGATGCGAGTGAT
TTTGATGACGAGCGTAATGGCTGGCCTGTTGAACAAGTCTGGAAAGAAATGCATAAGCTTTTGCCATTCTCACCGGATTCA
GTCGTCACTCATGGTGATTTCTCACTTGATAACCTTATTTTTGACGAGGGGAAATTAATAGGTTGTATTGATGTTGGACGAG
TCGGAATCGCAGACCGATACCAGGATCTTGCCATCCTATGGAACTGCCTCGGTGAGTTTTCTCCTTCATTACAGAAACGGCT
TTTTCAAAAATATGGTATTGATAATCCTGATATGAATAAATTGCAGTTTCATTTGATGCTCGATGAGTTTTTCTAA

Kanamycin ORF (*S. pombe* version)

ATGGGAAAAGAAAAAACTCATGTCTCTCGTCCTCGTCTGAACTCGAACATGGATGCTGATCTTTATGGTTATAAATGGGCTC
GTGATAATGTAGGACAATCAGGAGCAACAATATACAGACTCTACGGAAAGCCAGACGCTCCAGAATTATTTTTAAAACATGG
AAAAGGTTCTGTTGCTAATGATGTGACTGATGAGATGGTTCGTTTGAATTGGCTTACTGAATTTATGCCTTTACCTACTATTA
AACATTTCATAAGAACACCAGATGACGCATGGCTCCTCACAACAGCGATCCCAGGAAAAACAGCTTTTCAAGTTTTAGAAGA
ATATCCTGATTCTGGAGAGAACATTGTTGATGCTCTTGCTGTTTTTCTTCGTCGTTTACATTCTATACCTGTTTGTAACTGTCC
ATTCAATAGTGACAGAGTATTCAGACTCGCACAAGCGCAGAGCAGAATGAACAATGGATTAGTAGATGCTTCTGATTTTGAT
GATGAGAGAAATGGCTGGCCTGTTGAGCAGGTTTGGAAGGAGATGCATAAATTATTACCTTTTTCTCCTGATAGTGTAGTAA
CACATGGAGACTTCAGCCTAGACAACCTCATCTTCGACGAAGGAAAATTAATAGGATGTATAGATGTTGGAAGAGTTGGAA
TTGCTGATCGCTATCAGGATCTTGCGATTCTTTGGAATTGTCTTGGTGAGTTTAGTCCTTCTTTACAAAAAAGATTATTCCAA
AAATATGGAATAGACAACCCAGACATGAACAAGCTTCAATTTCATTTAATGTTAGATGAATTTTTTTAA

Kanamycin ORF (*S. cerevisiae* version)

ATGGGCAAGGAGAAGACCCACGTGTCCCGCCCCCGCCTGAACTCCAACATGGACGCGGACCTTTACGGTTATAAGTGGGC
TCGTGATAATGTCGGGCAATCAGGAGCAACAATCTACAGGCTCTACGGCAAGCCCGACGCGCCGGAGCTGTTCCTGAAGC
ACGGCAAGGGCAGTGTGGCCAACGACGTGACCGACGAGATGGTGCGGCTGAACTGGCTGACGGAGTTCATGCCTCTTCCT
ACTATTAAACATTTTATCCGTACTCCAGATGATGCATGGCTGCTCACAACCGCCATCCCCGGCAAGACGGCGTTCCAGGTG
CTGGAGGAGTACCCGGACAGTGGGGAGAACATCGTGGACGCCCTGGCGGTGTTCCTGCGCCGGCTGCACTCGATTCCTGT
TTGTAATTGTCCTTTTAACAGCGATCGCGTATTTAGATTAGCACAAGCGCAAAGCCGCATGAACAACGGCCTCGTCGACGC
CAGCGACTTCGACGACGAGCGCAACGGGTGGCCGGTGGAGCAGGTGTGGAAGGAGATGCACAAGCTGCTGCCGTTTTCT
CCTGATTCTGTTGTTACTCATGGTGATTTCTCATTAGATAATCTAATCTTCGACGAAGGCAAGCTCATCGGCTGCATCGACG
TCGGCAGGGTGGGCATCGCGGACAGGTACCAGGACCTGGCCATCCTGTGGAACTGCCTGGGGGAGTTCTCGCCGTCGCT
TCAGAAGCGTCTTTTTCAAAAATATGGTATTGATAATCCAGATATGAACAAACTGCAATTCCACCTCATGCTCGACGAGTTCT
TCTGA

Supplemental Figure S1. Wild type and modified DNA sequences used in this work

S1A) Sequences corresponding to Figure 1. Wild type and modified versions of *S. pombe ura4* ORF. Initiation and termination codons (ATG and TAA) are underlined. Sequences harbouring synonymous codons relative to the wild type ORF are indicated in red. The four ORFs have the same size (795 bp) and encode the same Ura4 protein.

S1B) Sequences corresponding to Figure 2. Wild type and randomized sequences underlying nucleosomes 3-4. The genomic position of the first and last nucleotide of the wild type sequence is indicated.

S1C) Sequences corresponding to Figure 3. Sequence and genomic position of the six fragments used to assemble the chimaeric sequence and the sequence resulting from the randomization of the six individual sequences.

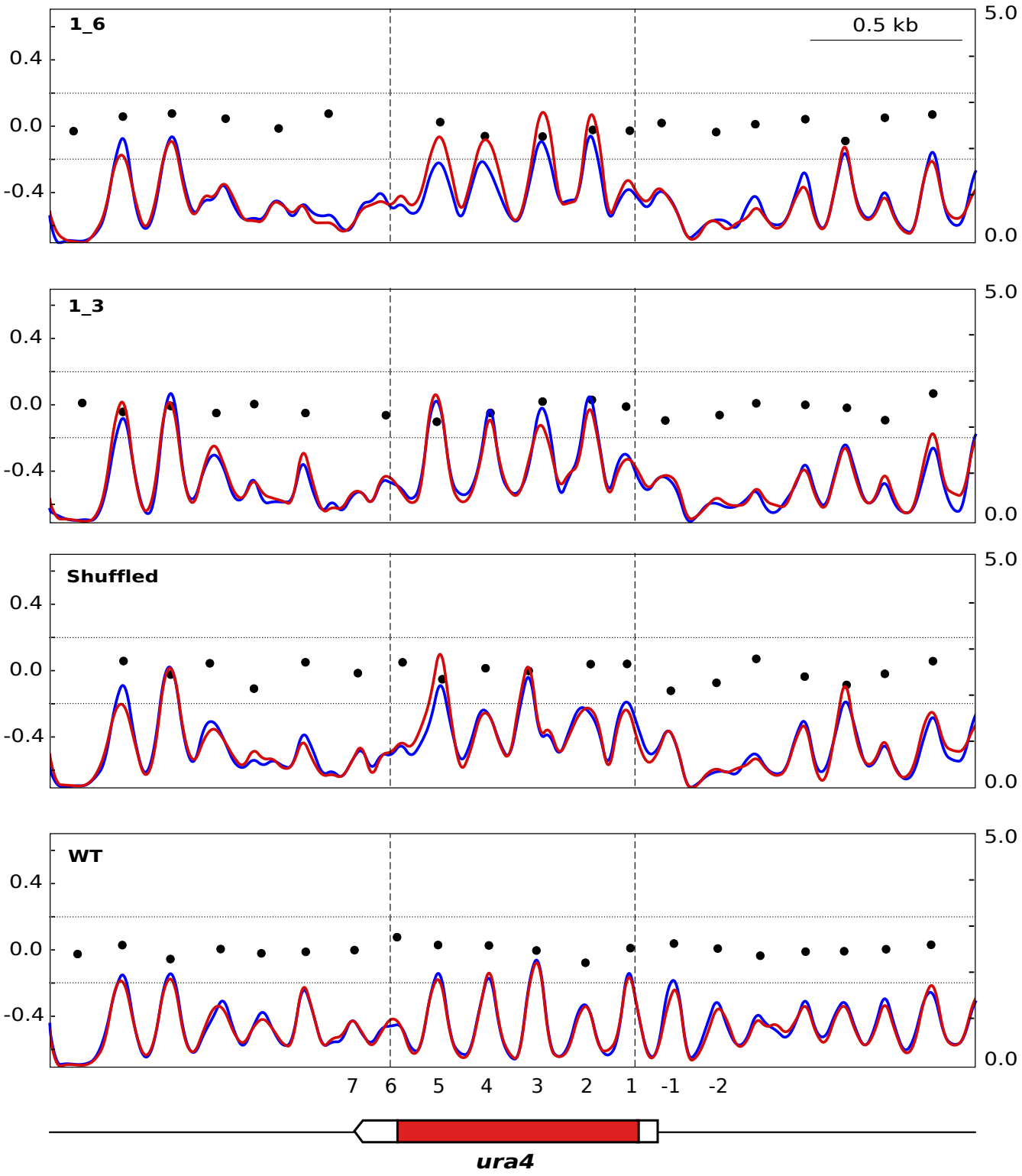
S1D) Sequences corresponding to Figure 4. Sequences harbouring synonymous codons relative to the wild type *S. pombe ura4* ORF in the dyad, linker or internal regions of mononucleosomal DNA are indicated in red. The three ORFs have the same size (795 bp) and encode the same Ura4 protein.

S1E) Sequences corresponding to Figure 6. Sequence of the synthetic molecules designed to position nucleosomes in *S. pombe* and in *S. cerevisiae*.

S1F) Sequences corresponding to Figure 7. Native and remastered *ura4* ORFs of *S. octosporus and S. japonicus*. In the modified versions, the wild type codons have been replaced by their synonymous codons following the nucleosomal signature of *S. pombe*. The four sequences have the same size (975 bp) and encode the Ura4 proteins of *S. octosporus* or *S. japonicus*.

S1G) Sequences corresponding to Figure 8. Wild type and modified sequences of the *kanamycin* ORF. In the modified versions, the wild type codons have been replaced by their synonymous codons following the nucleosomal signature of *S. pombe* or *S. cerevisiae*. The three sequences have the same size (810 bp) and encode the same Kanamycin resistance protein.

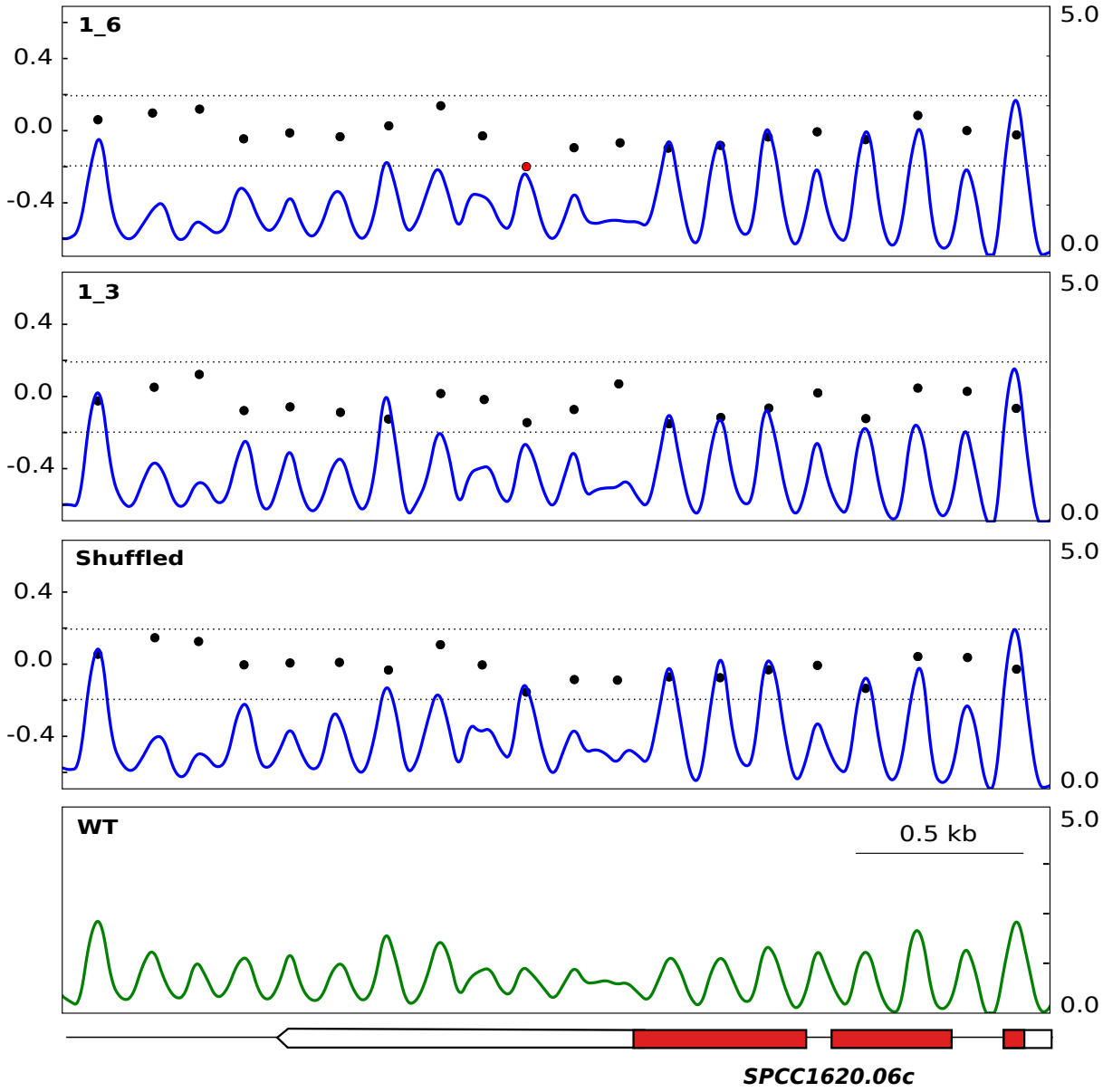
Supplemental Figure S2



Variation in the pattern of nucleosome occupancy between biological replicates of the same strains.

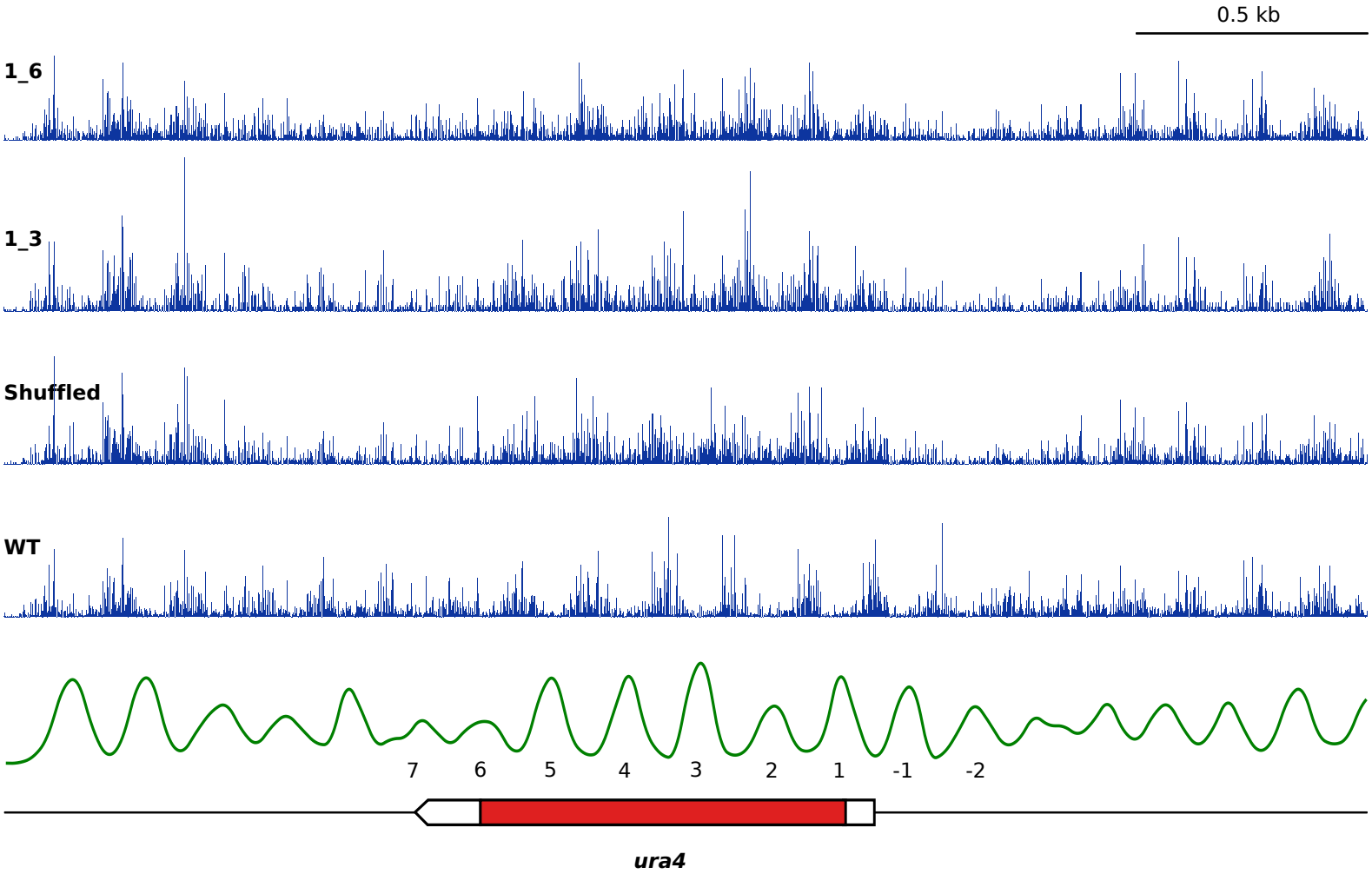
Nucleosomal occupancy profiles of the *ura4* region of biological replicates (red and blue) of the four *S. pombe* strains shown in Figure 1. Black dots show the log2 value of the differences between each pair of replicates as measured by DANPOS (see text and Methods for details). Y-axis scales are as in Figure 1D.

Supplemental Figure S3



Variation in the pattern of nucleosome occupancy of endogenous, non-modified regions between different strains.
Blue and green traces represent the average nucleosomal occupancy of the *SPCC1620.06C* region of the two biological replicates of each strain shown in Figure 1. Symbols and scales are as in Figure 1D.

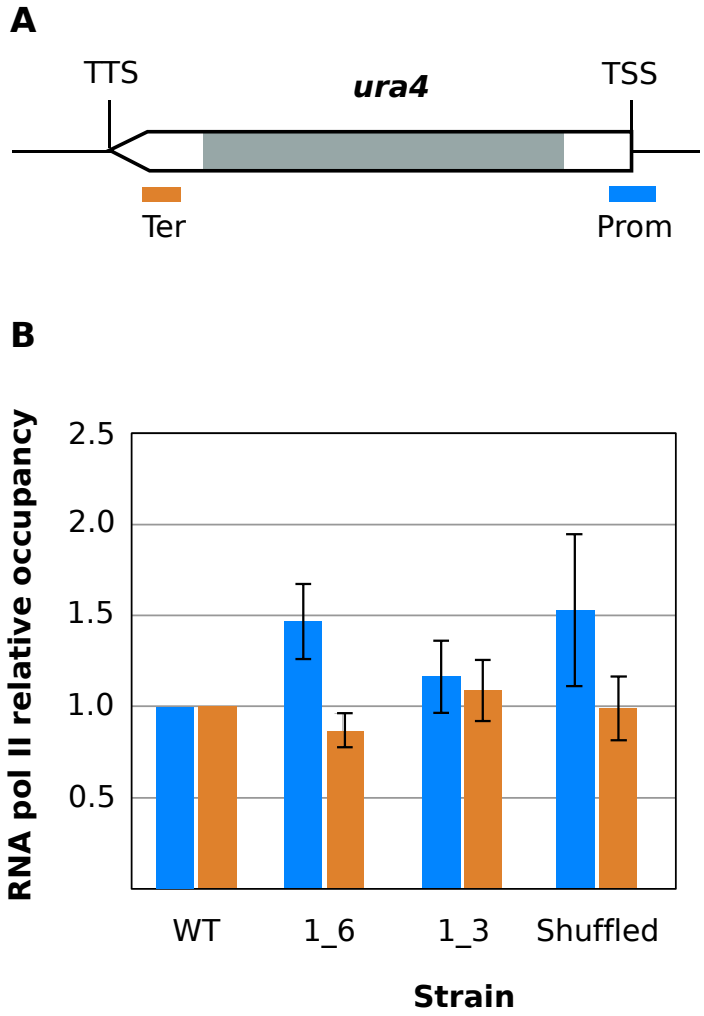
Supplemental Figure S4



Distribution of MNase cutting sites along the *ura4* ORF

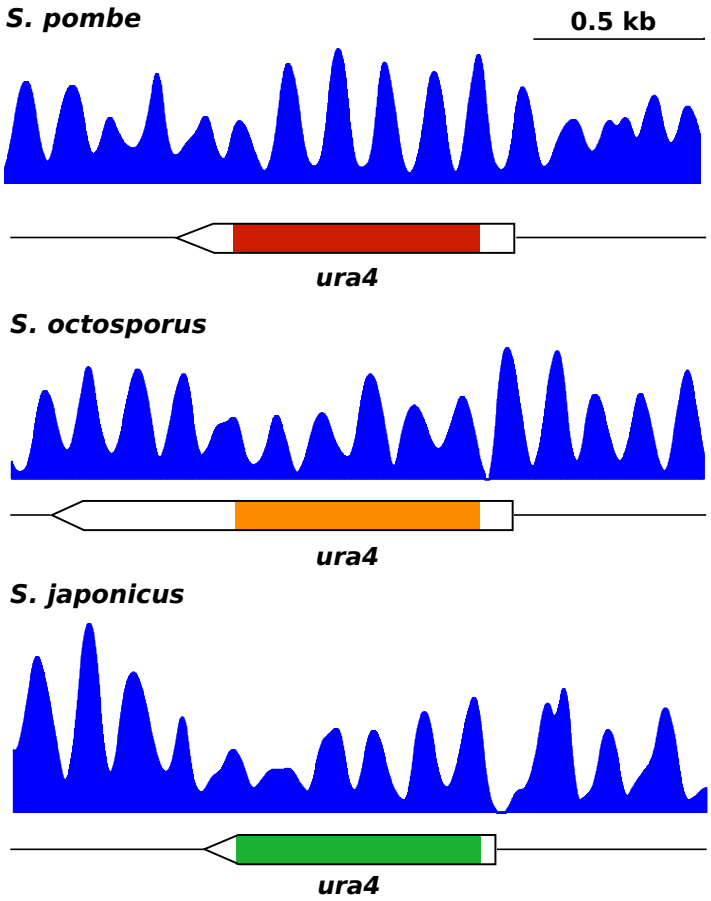
The MNase-seq profile and the positions of nucleosomes -2 to 7 shown in Figure 1 is represented at the bottom (green trace). The ORF of the *ura4* gene is shown in red. The distributionof cutting sites of MNase (vertical lines) is concentrated within internucleosomal linker regions in wild type cells but it is more dispersed along the ORF in the 1_6 and Shuffled strains, consistent with the end-labelling mapping shown in Figure 1. A lower degree of dispersion is also detectable in the 1_3 strain.

Supplemental Figure S5



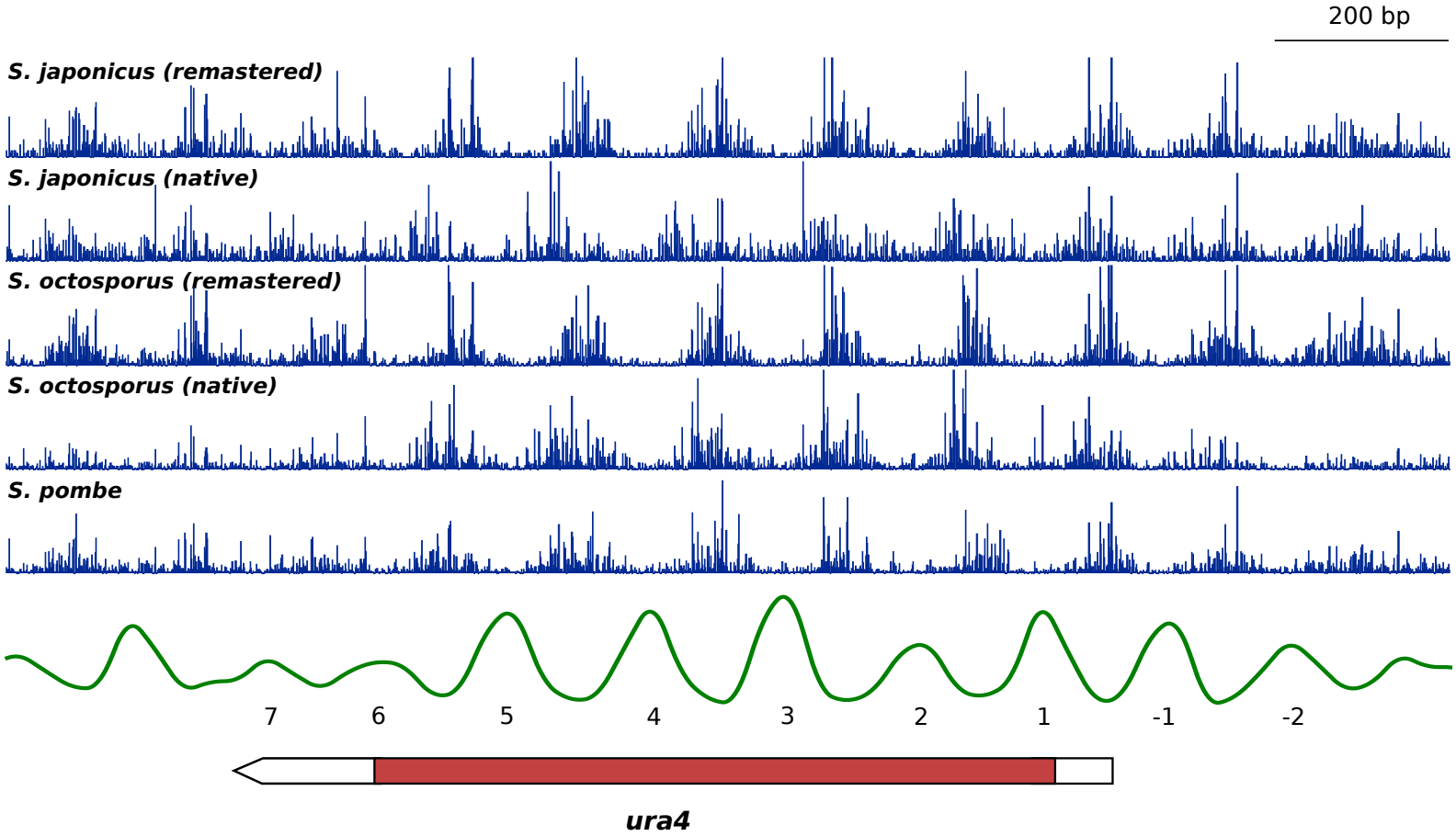
RNA polymerase II occupancy along wild type and modified *S. pombe ura4* gene
A) Diagramme of the *ura4* gene. The transcription start (TSS) and transcription termination (TTS) sites are indicated. The ORF is shown in grey. One of the regions tested by qPCR maps to the 3' end of the transcript (orange bar, Ter) and the other encompasses the TSS (blue bar, Prom). The two regions are present in all the strains with modified ORFs studied in this work. B) Sonicated chromatin was immunoprecipitated with an anti RNA pol II antibody and the abundance of the Prom and Ter regions was measured by qPCR. Results of the strains in Figure 1 were normalized to an internal control at the *mat* locus and then referred to the strain harbouring the WT version of the *ura4* ORF. The standard deviation of 3 independent experiments is shown.

Supplemental Figure S6



Nucleosomal distribution across the ura4 region of fission yeasts
Nucleosome occupancy maps for *S. octosporus* and *S. japonicus* are taken from MNase-seq data from Quintales et al. (2015a). The ORF of the three *ura4* genes of *S. pombe*, *S. octosporus* and *S. japonicus* is shown in red, orange and green, respectively.

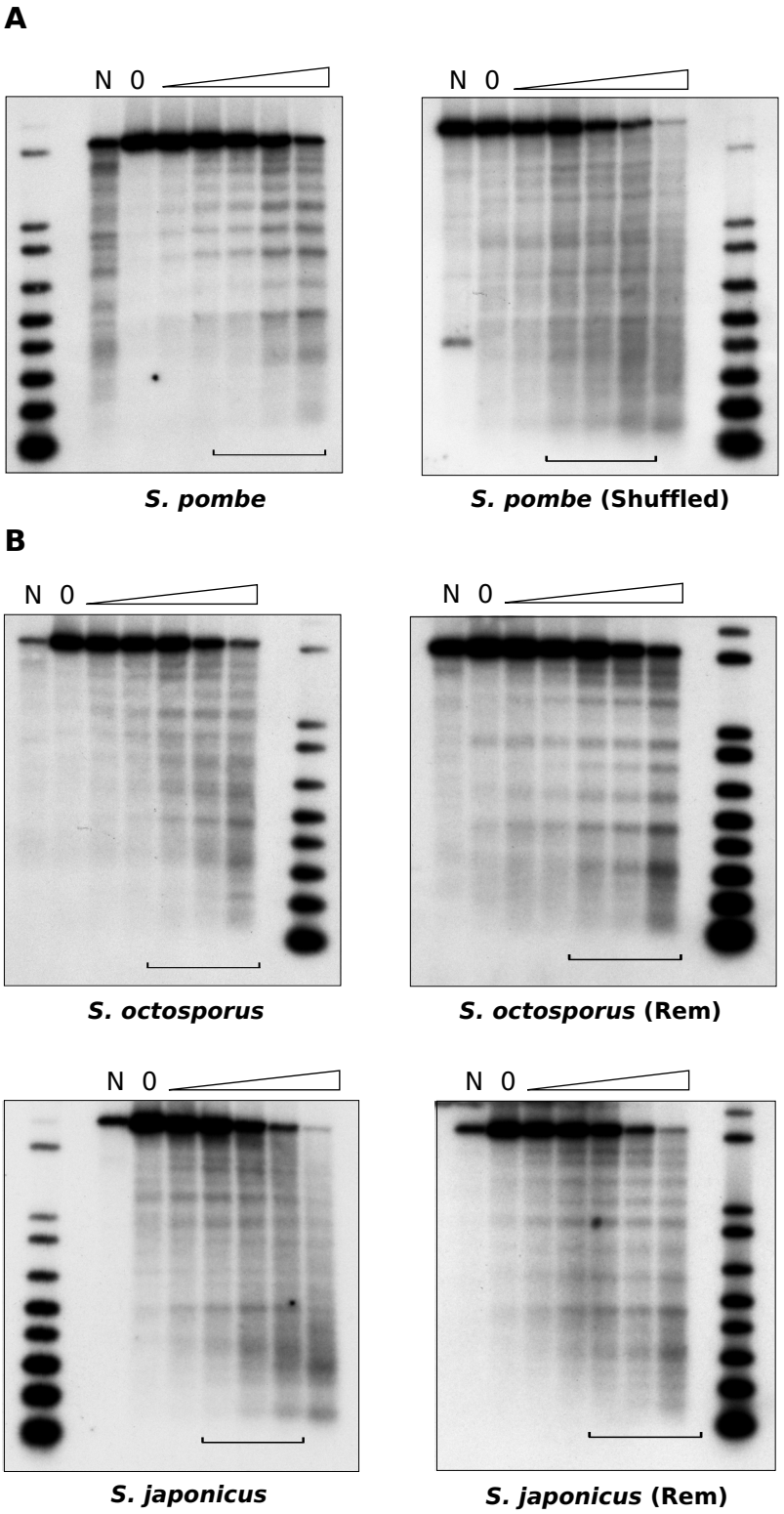
Supplemental Figure S7



Distribution of MNase cutting sites along orthologous *ura4* ORFs

The position of nucleosomes -2 to 7 in Figure 7B are represented at the bottom. The distribution of cutting sites of MNase (vertical lines) is concentrated within internucleosomal linker regions in the *S. pombe ura4* ORF. The distribution is slightly more dispersed in the *S. octosporus* native ORF and much more in the *S. japonicus* native ORF. By contrast, in the *S. octosporus* and *S. japonicus* remastered ORFs, MNase cutting sites concentrate at the same regions as in the *S. pombe* ORF, coinciding with the MNase end-labelling mapping in Figure 7A.

Supplemental Figure S8



Micrococcal nuclease analysis of wild type and modified *S. pombe* sequences
A) Chromatin from *S. pombe* wild type cells (WT) and from the *S. pombe* Shuffled strain were digested with increasing amounts of MNase (indicated with triangles, see Methods) prior to digestion with the appropriate restriction enzymes as in Figure 1. Lanes N contain naked DNA digested with MNase and lanes 0 contain chromatin incubated without MNase under the same conditions as the five samples treated with the enzyme. Brackets at the bottom of the panels indicate the three gel lanes shown in Figure 1A. B) Same analysis of the *S. pombe* strains harbouring the *S. octosporus* or the *S. japonicus* native or remastered *ura4* ORF shown in Figure 7A.