

Table S1. Summary of Sequencing Datasets, Related to Figure 1

| HTP-tagged yeast strain | Total reads after duplicates collapsed | Mapped reads | Mapped reads after second collapsing step | Reads spanning Intron-Exon or Exon-Exon junctions | Hits in Pol II transcribed RNAs |
|-------------------------|--|--------------|---|---|---------------------------------|
| Untagged | 132,540 | 104,828 | NA | Not analysed | 47619 |
| | 3,279 | 1,945 | NA | Not analysed | 343 |
| | 177,394 | 105,339 | 38,377 | Not analysed | 19801 |
| | 64,648 | 49,122 | 13,866 | Not analysed | 6599 |
| Hek2 | 1,203,569 | 684,274 | 365,358 | 1318 | 326077 |
| Gbp2 | 3,384,410 | 1,747,127 | 1,006,460 | 7375 | 730902 |
| Hrp1 | 681,331 | 573,431 | NA | 1663 | 345972 |
| | 819,092 | 577,177 | NA | 2257 | 351900 |
| Mex67 | 280,324 | 231,026 | NA | 336 | 114809 |
| | 311,754 | 247,326 | NA | 323 | 121804 |
| | 530,155 | 346,093 | 177,812 | 481 | 82351 |
| Mtr4 | 1,005,308 | 730,591 | 208,427 | 1013 | 63931 |
| | 2,035,764 | 1,514,999 | 393,099 | 2407 | 142513 |
| | 3,783,833 | 2,591,016 | 988,042 | 3971 | 453470 |
| Nab2 | 449,579 | 372,485 | 285,723 | 1866 | 200446 |
| | 6,479,021 | 4,823,691 | 2,340,092 | 22455 | 1715833 |
| | 1,452,862 | 1,058,178 | 475,551 | 8106 | 319860 |
| Nab2 Δ rrp6 | 3,413,860 | 2,307,904 | 1,307,646 | 10283 | 943578 |
| Pab1 | 31,828,181 | 24,433,808 | 6,299,244 | 25820 | 5233431 |
| Ski2 | 1,016,310 | 819,307 | 213,604 | 1604 | 120541 |
| | 1,351,395 | 1,044,648 | 674,270 | 2652 | 485147 |
| | 342,397 | 247,038 | 150,355 | 517 | 87686 |
| | 427,802 | 307,694 | 179,033 | 655 | 111144 |
| Cbc1 | 1,436,824 | 646,108 | 316,953 | 5267 | 242804 |
| | 1,056,734 | 618,448 | 498,142 | 5687 | 403414 |
| | 364,865 | 286,451 | 197,623 | 2117 | 137040 |
| Tho2 | 744,315 | 543,570 | 149,018 | 1427 | 83855 |
| Tif1 | 201,342 | 116,724 | NA | 1531 | 73963 |
| Trf4 | 313,153 | 247,673 | NA | 336 | 91761 |
| Xrn1 | 518,797 | 406,833 | NA | 1061 | 288562 |
| | 343,514 | 249,924 | NA | 623 | 175192 |

For all libraries, identical reads were collapsed to remove PCR duplicates (“Total reads after duplicates collapsed”). For libraries with 5’ adapters containing a random 3 nt barcode, additional duplicates were removed post-mapping, by collapsing reads with the same 3 nt barcode and 5’ genomic coordinate. For libraries constructed with 5’ adapters that do not contain the random 3 nt barcode, this second collapsing step could not be performed (indicated by “NA”). “Reads spanning Intron-Exon or Exon-Exon junctions” indicates the subset of reads used for analysis in Figure 1B. “Hits in Pol II transcribed RNAs” indicates the total number of reads that map to mRNAs, CUTs, SUTs, snRNAs and snoRNAs, and this value was used for internal normalization of datasets in Figure 1.

Table S2. Number of mRNAs, CUTs, and SUTs in Each Cluster, Related to Figure 2

| Cluster | mRNAs | | | CUTs | | SUTs | |
|---------|-------|----------------|-----------------------------|-------|---------------|-------|---------------|
| | Count | % of all mRNAs | Intron-containing pre-mRNAs | Count | % of all CUTs | Count | % of all SUTs |
| I | 100 | 2.5 | 0 | 310 | 55.4 | 69 | 15.9 |
| II | 190 | 4.8 | 5 | 76 | 13.6 | 85 | 19.6 |
| III | 121 | 3.1 | 1 | 141 | 25.2 | 131 | 30.2 |
| IV | 441 | 11.1 | 12 | 11 | 2.0 | 51 | 11.8 |
| V | 219 | 5.5 | 66 | 10 | 1.8 | 42 | 9.7 |
| VI | 532 | 13.4 | 55 | 0 | 0.0 | 8 | 1.8 |
| VII | 498 | 12.6 | 7 | 2 | 0.4 | 17 | 3.9 |
| VIII | 695 | 17.5 | 12 | 1 | 0.2 | 15 | 3.5 |
| IX | 590 | 14.9 | 23 | 5 | 0.9 | 9 | 2.1 |
| X | 580 | 14.6 | 32 | 4 | 0.7 | 7 | 1.6 |

We also include the number of intron-containing (pre-)mRNAs in each cluster.