Supplementary methods

1. *Generation of genomic libraries and sequencing*

Chromosome DNA was extracted from the lager brewing yeast and purified by CsCl-EtBr density gradient centrifugation following the methods reported previously.[1] A shotgun clone library of small inserts was constructed in the vector pUC18 (Amersham Biosciences).[2] The DNA was mechanically sheared into 1.5- to 2.5-kb fragments using a Hydroshear apparatus (Gene-machines) to prepare a shotgun clone library. Small fragments (<500 bp) were removed with Spin columns (Amersham-Pharmacia). The DNA fragments were blunt-ended and phosphorylated using a BKL kit (Takara) and cloned into the dephosphorylated *Sma*I site of the pUC18 vector (Amersham-Pharmacia). A library with large inserts was constructed in the cosmid vector Supercos-1 as follows. Chromosomal DNA was partially digested with *Sau*3AI to a mean size of 40–60 kb, ligated to Supercos-1, and packaged into λ phage particles using a packaging extract (Stratagene) as recommended by the manufacturer.

Sequencing both ends of the inserts of the plasmid and cosmid clones was performed by the Dye-terminator cycle sequencing method using MegaBACE3000 and ABI3700 as previously described.[3] A total of 331,798 sequences were obtained by sequencing of the shotgun clone and the cosmid clone libraries. Initially, the 12,304 sequences

from 6,152 cosmid clones were aligned against the 16 chromosomes of *S. cerevisiae* (SGD) using NCBI-BLAST blastn ver. 2.0.7 (parameters: W 7, r 17, q 21, f 280, G 29, E 22, X 240), and the longest alignments were considered as significant. We roughly identified the sequences that had more than 94% DNA identity to the corresponding sequence of *S. cerevisiae* as Sc-type sequences and the other sequences as Non-Sc-type sequences and mapped them on the *S. cerevisiae* 16 chromosomes according to the result of the blast. The cosmid clones having both end sequences identified as the Sc-type, those having both end sequences identifid as the Non-Sc-type, and those having one end sequence identified as the Sc-type and the other as the Non-Sc-type were considered to be Sc-type, Non-Sc-type, and Sc/Non-Sc-type cosmid clones, respectively. A set of 338 Non-Sc-type cosmid clones that were overlapping on 16 Non-Sc-type chromosomes was selected to make cosmid subclone libraries (Supplementary Table S1). Eight additional cosmids were chosen for making subclone libraries, since each had an Sc-type sequence at one end and an Non-Sc-type at the other end, making these cosmids candidates for containing breakpoints of recombination between the Sc- and Non-Sc-type sub-genomes (Supplementary Table S1).

Cosmid subclone libraries were constructed in the vector pSFI-CV as previously described.[4] The cosmid was partially digested with *Sfi*I, and the 2-6 kb fragments

obtained by gel electrophoresis were cloned into pSFI-CV. Sequencing both ends of each plasmid clone from the selected cosmid clone with a 2-3× coverage was performed by the Dye-terminator cycle sequencing method as described above, and 97,769 sequences were obtained.

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2. Yanisch-Perron, C., Vieira, J., and Messing, J. 1985, Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. *Gene.*, **33**, 103-119.
3. Toyoda, A., Noguchi, H., Taylor, T.D. et al. 2002, Comparative genomic sequence analysis of the human chromosome 21 Down syndrome critical region. *Genome Res.*, **12**, 1323-1332.
4. Hattori, M., Tsukahara, F., Furuhata, Y. et al. 1997, A novel method for making nested deletions and its application for sequencing of a 300 kb region of human APP locus. *Nucleic Acids Res.*, **25**, 1802-1808.