A strategy for constructing aneuploid yeast strains by transient nondisjunction of a target chromosome

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Abstract

Background: Most methods for constructing aneuploid yeast strains that have gained a specific chromosome rely on spontaneous failures of cell division fidelity. In Saccharomyces cerevisiae, extra chromosomes can be obtained when errors in meiosis or mitosis lead to nondisjunction, or when nuclear breakdown occurs in heterokaryons. We describe a strategy for constructing N+1 disomes that does not require such spontaneous failures. The method combines two well-characterized genetic tools: a conditional centromere that transiently blocks disjunction of one specific chromosome, and a duplication marker assay that identifies disomes among daughter cells. To test the strategy, we targeted chromosomes III, IV, and VI for duplication.

Results: The centromere of each chromosome was replaced by a centromere that can be blocked by growth in galactose, and ura3::HIS3, a duplication marker. Transient exposure to galactose induced the appearance of colonies carrying duplicated markers for chromosomes III or IV, but not VI. Microarray-based comparative genomic hybridization (CGH) confirmed that disomic strains carrying extra chromosome III or IV were generated. Chromosome VI contains several genes that are known to be deleterious when overexpressed, including the beta-tubulin gene TUB2. To test whether a tubulin stoichiometry imbalance is necessary for the apparent lethality caused by an extra chromosome VI, we supplied the parent strain with extra copies of the alpha-tubulin gene TUB1, then induced nondisjunction. Galactose-dependent chromosome VI disomes were produced, as revealed by CGH. Some chromosome VI disomes also carried extra, unselected copies of additional chromosomes.

Conclusion: This method causes efficient nondisjunction of a targeted chromosome and allows resulting disomic cells to be identified and maintained. We used the method to test the role of tubulin imbalance in the apparent lethality of disomic chromosome VI. Our results indicate that a tubulin imbalance is necessary for disomic VI lethality, but it may not be the only dosage-dependent effect.
Background
Any change in chromosome number through the gain and/or loss of part of a haploid set of chromosomes is known as aneuploidy. Aneuploidy leads to defects in the growth and development of an organism (reviewed in [1,2]). In cases of chromosome gain, the phenotype of an aneuploid is influenced by the effects of two phenomena: (1) a general, physiological response to excess protein expression, leading to a slowing of cell proliferation [3], and (2) protein stoichiometry imbalances specific to specific aneuploid karyotype requires an ability to manipulate standing of the complex phenotype caused by any specific genes on the extra chromosome [1-3]. A complete understanding of the complex phenotype caused by any specific genes on the extra chromosome [1-3]. A complete understanding of the complex phenotype caused by any specific genes on the extra chromosome [1-3]. A complete understanding of the complex phenotype caused by any specific genes on the extra chromosome [1-3]. A complete understanding of the complex phenotype caused by any specific genes on the extra chromosome [1-3].

In the yeast Saccharomyces cerevisiae, aneuploids have been isolated in a number of ways over the years. Strains with extra chromosomes have arisen spontaneously among lab strains (for examples, see [4,5]), and have been generated through meiosis of triploids [6]. Specific disomes (haploids carrying an extra chromosome, karyotype N+1) have been isolated by differentially marking two homologs in a diploid, then selecting for meiotic segreants that contain both homologs (for example, [7]). An alternative method to generate disomes makes use of transient heterokaryons that form during mating between kar1 and KAR1+ haploids [8]. At a certain frequency, chromosomes are transferred from one nucleus to another before one nucleus is lost. By differentially marking homologs in the parents and selecting for progeny cells that retain both homologs, haploid progeny carrying a disomic chromosome have been isolated [9,10]. This method, termed chromoduction, was used to select for 14 of the possible 16 disomes of yeast in a recent systematic study of aneuploidy [3]. Although the methods described above are clearly effective at isolating disomic strains of yeast, each of them requires a spontaneous failure of chromosome segregation during cell division. The mechanisms that underlie these failures (the breakdown of nuclear integrity in a cell containing multiple nuclei or the bypass of the spindle assembly checkpoint to allow nondisjunction [11,12]) are not well understood, and may lead to additional, unplanned genetic changes.

We have devised a method for generating disomes that does not rely on spontaneous failure in cell division integrity. Instead, the method specifically blocks mitotic segregation of the target chromosome alone. The method comprises a novel combination of two well-characterized genetic tools, a conditional centromere [13] and a duplication marker [14]. When these are placed at the centromere of a target chromosome, disjunction of the chromosome can be transiently blocked to generate disomic cells, some of which are selectively identified by the duplication marker.

We report the results of a proof-of-concept test with chromosomes III, IV, and VI. The method efficiently generated disomic III and IV strains, but did not produce disomic VI unless TUB1 copy number was also increased.

Results and discussion
General strategy to induce and select for a duplicated chromosome
The strategy involves modifying the centromeric region of a target chromosome so that (1) the centromere can be inactivated temporarily to cause nondisjunction and (2) daughter cells that obtained two copies of the target chromosome can be selected. The chromosome modification strategy is outlined in Figure 1A and 1B. The conditional centromere construct P\textsubscript{GAL1}-CEN3 URA3 [13] is PCR-amplified with primers that provide homology to sequence flanking the target centromere. The PCR fragment is transformed into yeast and integrated into the target site by homologous recombination, replacing the endogenous centromere [15] (Figure 1A). P\textsubscript{GAL1}-CEN3 functions as an autonomous centromere when placed into plasmids or chromosomes, and its function can be blocked when galactose induces GAL1 promoter activity [13,16]. In galactose, many kinetochore proteins do not assemble on the centromere, but within 20 minutes of a switch to glucose, kinetochore assembly is observed [17]. Once the conditional centromere is in place, a marker that can detect changes in ploidy is generated at URA3, following a strategy devised by Chan and Botstein [14]. A plasmid containing HIS3 and an internal fragment of \textit{ura3} is transformed into yeast, integrating into \textit{URA3} and disrupting its function (Figure 1B, forward arrow). Chromosome duplication is detected based on the properties of this integrated plasmid. The integration creates a 390 bp direct repeat of a portion of \textit{ura3}. Homologous recombination between the repeats causes excision and loss of HIS3 and regeneration of functional URA3 (Figure 1B, reverse arrow). (For simplicity, we refer to this event as excision of HIS3 even though it can occur by a variety of homologous recombination events including excision, gene conversion and unequal sister chromatid exchange [18].) Because HIS3 can be lost and URA3 regenerated, the locus exists in one of two mutually exclusive states: either it will contain an intact HIS3 or an intact URA3, but not both. Duplication of this marker locus, followed by excision of HIS3, should lead to cells that contain both URA3 and HIS3. Such cells should exhibit Ura+His+ phenotypes.

The strategy for inducing and selecting disomic cells is outlined in Figure 1C. A haploid strain with a chromosome containing the conditional centromere and duplication marker is exposed to galactose during one cell division cycle. The conditional centromere is inactivated and the target chromosome fails to disjoin during mitosis. Among the daughter cells that contain two copies of the...
target chromosome, a fraction will spontaneously excise one of the HIS3 markers to generate URA3. These cells will be recovered as Ura+His+ colonies on selective medium.

Construction of modified chromosomes as targets for duplication
As a proof-of-concept test, we chose three chromosomes (III, IV, and VI) to modify and target for duplication. Chromosomes III and VI are among the smallest yeast chromosomes (317 and 270 kb, respectively), whereas chromosome IV is the one of the largest (1.53 Mb) [19]. We anticipated that chromosomes IV and VI would pose a challenging test of this method because they were among the least frequently isolated disomes using chromoduction [10], and disomic strains containing a single extra chromosome VI failed to be isolated by Torres et al. [3]. Rather, colonies selected for chromosome VI disomy also contained extra, unselected chromosomes, suggesting that simple chromosome VI disomes may be inviable [3]. We constructed each modified chromosome twice, independently, and tested for concordance of phenotype. The chromosomes were constructed in diploids (or in haploids that were subsequently mated to wild type) to produce cells heterozygous for the conditional centromere and duplication markers. When the heterozygotes were sporulated, the modified chromosomes segregated 2:2 and produced haploid colonies on rich medium that were indistinguishable from wild-type segregants (Additional file 1A). We conclude that the centromeric modifications themselves do not lead to growth phenotypes.

To test whether the conditional centromeres in our strains could be inhibited by galactose to cause nondisjunction, we tested for chromosome loss by constructing diploid strains that were heterozygous for a conditional centromere marked with URA3. Growth in galactose, followed by plating to glucose-containing medium, resulted in the appearance of many Ura- colonies. Consistent with Hill and Bloom's observations [13], galactose exposure for 1–2 generations led to the loss of the URA3 marker in approximately 50% of the cells (Additional file 1B). In the case of diploids carrying modified chromosome IV, most of the galactose-induced Ura- colonies exhibited a severe, slow-growth phenotype, suggesting that the Ura- colonies were the result of losing the target chromosome (Additional file 1C). We conclude that the conditional centromere allows for galactose-inducible nondisjunction in our strains.

To characterize the ura3::HIS3 duplication marker, we measured the frequency of HIS3 excision and reconstitution of URA3 at each modified centromere. Each marker
excised HIS3 to produce Ura+ papillae at a frequency near $10^{-4}$ (Table 1). Galactose did not alter this frequency. When cultures were placed under selection for Ura+His+ papillae, strains with two copies of the marker produced colonies at the frequency of HIS3 excision, whereas strains with one copy produced colonies at a much lower frequency (Figure 2). To produce these rare Ura+His+ papillae, the strains with a single marker had to undergo spontaneous duplication of the chromosome (or the marker itself) in addition to HIS3 excision. Under selection for this kind of duplication marker, Chan and Botstein found that most events (85%) were likely catastrophic increases in ploidy rather than single chromosome gains [14]. We conclude that the *ura3::HIS3* marker constructed here should identify cells that have gained an extra copy of the modified chromosome.

**Targeted chromosomes are duplicated after nondisjunction is induced**

If nondisjunction of the conditional centromere causes chromosome gain as well as chromosome loss, induced disomic cells should be detectable by an increase in the frequency of appearance of Ura+His+ colonies. To select directly for Ura+His+ disomes, haploid cells carrying a modified chromosome were grown to log phase in medium containing raffinose, the cultures were split, and galactose was added to one of the resulting cultures. After 1–1.3 culture doublings, cells were spread to selective plates. In the absence of galactose, all strains produced spontaneous Ura+His+ papillae at a frequency of approximately $10^{-6}$ (Figure 3). For strains containing modified chromosome III or IV, growth in galactose increased the frequency of Ura+His+ papillae formation (Figure 3A), suggesting that many of the papillae had developed from disomes that formed by galactose-induced nondisjunction. In contrast, the strains containing modified chromosome VI showed no increase in the appearance of Ura+His+ papillae when grown in galactose. (We consider this lack of chromosome VI duplication below.)

In addition to direct selection for Ura+His+ colonies, we tested whether a "delayed selection" scheme could identify candidate disomes (Figure 4A). Cells carrying a modified chromosome IV were grown in galactose for 1.3 culture doublings, diluted, and plated to rich medium. The resulting colonies were replica-plated to selective medium and screened for colonies that produce numerous Ura+His+ papillae (Figure 4B). Among colonies from 7 independent galactose-treated cultures, 4.8% behaved as candidate disomes, whereas only 0.37% of the colonies from cultures that were not exposed to galactose looked like candidate disomes (standard deviations were 2.8% and 0.43%, respectively).

To determine whether Ura+His+ isolates were disomic, we examined chromosome copy number by microarray-based comparative genomic hybridization (array CGH). Among galactose-induced cultures, we tested 3 isolates that targeted chromosome III and 6 that targeted IV (1 selected directly and 5 from the delayed selection protocol). All of these isolates contained a single, extra copy of the target chromosome (see Figure 5 for examples of each karyotype). In contrast, among spontaneous Ura+His+ isolates, 1/6 that targeted chromosome IV (1/3 direct selection, 0/3 delayed selection) displayed disomy. We conclude that this method, placing a conditional centromere and duplication marker on a target chromosome, allows for efficient isolation of newly formed disomic strains of yeast.

**Extra copies of TUB1 allow the isolation of chromosome VI disomes**

Since galactose exposure did not increase the frequency of Ura+His+ papillae in strains carrying a modified chromosome VI (Figure 3A), we did not have confidence that the selected colonies were disomic. Indeed, when 5 isolates were examined by array CGH, aneuploidy was not detected (data not shown). This result is consistent with the notion that chromosome VI disomy is lethal, as suggested by previous studies that found VI disomy to be either rare or absent [3,6,10,20]. For example, disomic haploids are frequently found among the rare, viable spores produced by interspecific hybrids of *Saccharomyces cerevisiae* and *paradoxus* [20]. Hunter et al. examined 300 such spores by CHEF gel analysis and observed no occur-

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**Figure 2**

Papillation pattern in strains that contain one or two copies of the duplication marker *ura3::HIS3*. *Ura3::HIS3* strains were grown overnight in YPD rich medium. $10^6$ and $10^4$ cells from each culture were spotted to plates containing selective media lacking uracil (-ura) or uracil and histidine (-ura, -his). Papillae were scored after 3 days. The strains shown are KAY579 (haploid), KAY626 (hemizygous diploid), and KAY625 (homozygous diploid).
Table 1: Frequency of Ura+ cells in haploid strains containing ura3::HIS3 integrated next to a centromere

| Chromosome containing ura3::HIS3 | Galactose exposure prior to selection | Frequency of Ura+ |
|----------------------------------|-------------------------------------|------------------|
| III                              | -                                   | 3.4 ± 3.2 × 10^-4 |
| IVa                              | -                                   | 1.0 ± 0.0 × 10^-4 |
| VIb                              | -                                   | 9.7 ± 1.8 × 10^-5 |
| VIb                              | +                                   | 1.3 ± 0.6 × 10^-4 |

Haploid cells were grown overnight in YPD rich medium. 10^6 cells were plated onto selective medium lacking uracil. Colonies were counted after 3 days. The mean frequency and standard deviation obtained from at least 4 independent cultures are shown.

Cultures were grown in supplemented minimal medium containing raffinose. Where indicated, galactose was added to 1.5% and culture was grown for 1.3 doublings prior to plating onto selective medium. Diluted cultures were also plated to YPD to score viable cell density. Ura+ frequency was computed per viable cell plated. A paired t-test indicates that the frequencies were not different (2-tailed, P > 0.1).

References of chromosome VI disomy among the nine chromosomes detectable by this method, consistent with a possible lethality of disomic chromosome VI [20]. Similarly, in a systematic study of chromoduction, Dutcher found that when chromosome VI disomy was selected, the frequency of its appearance was much lower than other chromosomes of its size, perhaps because there is strong selection against chromosome VI disomes [10].

More recently, Torres et al. used chromoduction to construct and study a nearly complete set of N+1 disomic strains [3]. However, when chromosome VI disomy was selected and the resulting cells were examined by array CGH, the unselected chromosomes I and XIII were also present. The absence of single chromosome VI disomes suggests that such a karyotype may be lethal, and that the presence of chromosomes I and XIII suppresses this lethality [1,3].

If chromosome VI disomy is lethal, expression of one or more genes on the extra chromosome VI may cause stoichiometry imbalances severe enough to prevent viability. There are several well-studied genes on chromosome VI that are known to be deleterious upon overexpression, including CDC14, ACT1 and TUB2 [21-25]. For example, TUB2, which codes for beta-tubulin, has been shown to be exquisitely dosage-sensitive. Overexpression of TUB2 causes lethality, even when a single, extra copy is integrated into a haploid genome [23]. This lethality can be suppressed by increased expression of alpha-tubulin in the cell, encoded by the chromosome XIII genes TUB1 and TUB3 [26,27]. The observations of Torres et al. support the idea that the extra dose of TUB2 contributes to the lethality of disomic chromosome VI: in the viable strains that are disomic for I, VI, and XIII, the extra copy of chromosome XIII can supply the cell with additional alpha-tubulin, eliminating the stoichiometry imbalance caused by the extra copy of TUB2 [3].

Clearly, a second copy of TUB2 in a haploid is sufficient to cause lethality, as demonstrated by the TUB2 integration experiments of Katz et al. [23]. But when the entire chromosome VI is duplicated, is the duplication of TUB2 necessary for the extra copy of chromosome VI to cause lethality? If it is, then eliminating the tubulin imbalance alone should allow for the viability of chromosome VI disomes. However, if additional chromosome VI genes cause dosage imbalances severe enough to prevent viability, then simply eliminating the tubulin imbalance should not suppress the lethality of disomic chromosome VI.

We used the chromosome duplication method described here to test whether the tubulin imbalance is necessary for the lethality of VI disomy. We supplied TUB1-containing plasmids to haploid strains carrying modified chromosome VI, then induced nondisjunction and selected for duplication of the ura3::HIS3 marker. In contrast to strains without excess TUB1, many Ura+His+ candidate disomes did appear after exposure to galactose when the cells harboured a TUB1 plasmid (Figure 3B). The effect occurred when TUB1 was carried on a low-copy CEN plasmid or on a high-copy 2-micron plasmid.

Most of the Ura+His+ isolates, although viable, produced slow-growing, tiny colonies (Additional file 2). We grew 9 isolates in liquid culture, extracted DNA and performed array CGH. Although one culture did not exhibit aneuploidy, the other 8 were disomic for chromosome VI: 3 isolates were simple disomes, 3 isolates also contained an extra, unselected chromosome XII, and 2 contained extra chromosomes II and XII (see Figure 5 for examples of each karyotype). Since a number of colonies were isolated that contained the single, extra chromosome VI, and since these were only isolated when extra copies of TUB1 were present, we conclude that minimizing (or eliminating) the effect of TUB2 overexpression allows for the viability of chromosome VI disomes. TUB2 overexpression is therefore essential for the inviability of chromosome VI disomy.

Although viable, the chromosome VI disomes exhibited growth defects (Additional file 2). We do not know whether these defects are the result of residual tubulin...
dosage problems, other gene-specific effects, or a combination of the numerous ways that aneuploidy affects phenotype [1]. Further, it is not clear what role, if any, the unselected chromosomes II and XII play. Since the aim of this report is to describe our method for manipulating chromosome copy number, a complete study of the basis for chromosome VI dosage phenotypes will be reported elsewhere.

Conclusion
We have described a new method for inducing and selecting disomic yeast strains which does not rely on spontaneous errors in chromosome segregation. The method allowed for efficient isolation of disomic strains carrying either chromosome III or IV. We used the method to test a specific dosage relationship between chromosome VI and the alpha-tubulin gene TUB1, and found that chromosome VI disomes could be isolated with this method when plasmid-borne TUB1 was present. Our proof-of-concept test was therefore successful for each of the three chromosomes we tested.
In principle, any strain that already contains a conditional centromere could be supplemented with a duplication marker and used to generate disomic aneuploids. Reid et al., for example, have generated conditional centromeres on all 16 chromosomes for inducing loss of heterozygosity [28,29], and these chromosomes could be further modified as described above for the induction and selection of disomes. The method should be useful in studies investigating the genetic basis of aneuploid phenotypes, and any study that wishes to efficiently duplicate a chromosome de novo.

**Methods**

**Media and genetic manipulations**

Standard methods were used for growth and genetic analysis of yeast [30], except that YPD medium was supplemented with 50 mg/l adenine sulfate and 20 mg/l uracil. Sporulation was induced as described [31]. Unless otherwise noted, carbon sources were supplemented to 2% (wt/vol) and cells were grown at 30°C in supplemented minimal media to maintain plasmids or unstable integrations. Cell density was determined using a hemacytometer.

**Construction of plasmids**

Plasmids are listed in Table 2 [13,26,32,33]. Oligonucleotides were designed with Primer3 [34] and are listed in Additional file 3. Standard methods of DNA manipulation were used, unless otherwise noted [30,35]. To construct the plasmid pKA52, a 390 bp fragment internal to *URA3* was amplified from pGALCEN-JC3-13 template DNA with the use of oligonucleotide primers *URA3*int_F and *URA3*int_R in a high-fidelity *Pfu* polymerase chain reaction (PCR) (Stratagene). This fragment was digested with *Bam*HI and *Eco*RI, then ligated into the *Bam*HI/*Eco*RI sites of pRS303. The plasmid pKA55 was constructed using homologous recombination in yeast [36] to replace the *URA3* marker of pRB326 with *LEU2*. A 3.7 kb *Sph*I/*Pvu*II fragment containing *LEU2* and flanking vector sequence was cut from pRB327, gel-purified with the Qiaquick gel extraction kit (Qiagen), then combined with *Sma*I-linearized pRB326 DNA to co-transform a *leu2*- yeast strain. pKA55 was recovered by isolating DNA from Leu+ transformants and transforming *E. coli* strain JM109. To confirm that pKA55 contained functional *TUB1*, it was transformed into a *tub1Δ* strain containing the *TUB1*-URA3 plasmid pRB326. Cells were grown in medium supplemented with uracil to allow loss of pRB326, plated to YPD, then replica-plated to medium selecting for *Leu*+. Colonies were identified that were *Leu*+*Ura*-, indicating that the sole source of *TUB1* was from pKA55.

**Construction of yeast strains**

Yeast strains used in this study are listed in Table 3. All strains were derived from the S288C-related BY4741 and FY strains [37,38]. KAY519, KAY530 and KAY587 were...
descended from a cross between BY4741 and the FY3-derived DBY10147.

Haploid yeast strains were constructed to contain a modified chromosome (III, IV, or VI) that harbors a conditional centromere and a set of duplication markers (Figures 1A, B and Additional file 4). To construct KAY418 and KAY419, which carry a modified chromosome III, DNA containing PGAL1-CEN3 URA3 from plasmid pGALCEN-JC3-13 was transformed into strains DBY8923 and DBY8869, respectively, replacing chromosomal CEN3 as described [13]. To generate the ura3::HIS3 duplication marker, these strains were transformed with the integrative plasmid pKA52, which had been cut at the unique StuI site within its URA3 fragment. The strains were then backcrossed. Genetic linkage to chromosome III markers and centromere confirmed the CEN3 location of the integrated DNA. To generate KAY541 and KAY542, the strains were crossed to KAY530 to replace ura3-52 with ura3::HIS3 as described below.

To construct KAY614 and KAY619, which carry a modified chromosome IV, a 2.6 kb fragment containing PGAL1-CEN3 URA3 was amplified with high-fidelity *Phusion* PCR (Finnzymes) using the primers CEN4_REPL_F and CEN4_REPL_R and pGALCEN-JC3-13 template DNA. Each primer contains at its 5' end a 50 nt sequence identical to that found adjacent to CEN4. The PCR fragment was gel-purified (Qiaquick gel extraction kit, Qiagen) and used to transform the diploid strain DBY8869 × DBY8925. An independently-amplified fragment was used to transform the isogenic diploid DBY8871 × DBY8923. Each resulting strain was transformed with Stul-digested pKA52 to generate ura3::HIS3. To confirm that the conditional centromere integrated at CEN4, DNA from Ura His⁺ transformants was amplified by PCR using the primers CEN4_F and pGALCEN-JC3-13 template DNA. 

Table 2: Plasmids used in this study

| Plasmid       | Genotype       | Source                          |
|---------------|----------------|---------------------------------|
| pGALCEN-JC3-13| PGAL1-CEN3, URA3 | [13]                            |
| pRS303        | HIS3           | [32]                            |
| pRS315        | LEU2, CEN6, ARSH4 | [32]                           |
| pRS425        | LEU2, 2 μ      | [33]                            |
| pRS326        | TUB1, URA3, CEN4, ARS1 | [26]                         |
| pRS327        | TUB1, LEU2, 2 μ | [26]                            |
| pKA52         | HIS3, ura3 (390 bp fragment) | This study                  |
| pKA55         | TUB1, LEU2, CEN4, ARS1 | This study                |

(table continues)

Table 3: Yeast strains used in this study

| Strain       | Genotype       | Source                          |
|--------------|----------------|---------------------------------|
| BY4741       | MATα his3Δ1 leu2Δ10 met15Δ10 ura3Δ10 | [38]                           |
| DBY10147     | MATα           | D. Botstein (Princeton University) |
| DBY8869      | MATα his3Δ1200 ura3Δ52 | D. Botstein (Princeton University) |
| DBY8871      | MATα his3Δ1200 ura3Δ52 | D. Botstein (Princeton University) |
| DBY8923      | MATα his3Δ1200 ura3Δ52 ade2Δ1 leu2Δ1 lys2Δ1 | D. Botstein (Princeton University) |
| DBY8925      | MATα his3Δ1200 ura3Δ52 ade2Δ1 leu2Δ1 lys2Δ1 | D. Botstein (Princeton University) |
| KAY519       | MATα leu2Δ10  | This study                       |
| KAY530       | MATα his3Δ1200 ura3Δ10 | This study               |
| KAY537       | MATα his3Δ1200 leu2Δ10 met3Δ10 ura3Δ10 | This study               |
| KAY418, 419  | MATα his3Δ1200 ura3Δ52 cen3ΔPGAL1-CEN3 ura3Δ1:HI33(at CEN3) | This study               |
| KAY541, 542  | MATα his3Δ1200 ura3Δ10 cen3ΔPGAL1-CEN3 ura3Δ1:HI33(at CEN3) | This study               |
| KAY614, 619  | MATα his3Δ1200 ura3Δ10 cen3ΔPGAL1-CEN3 ura3Δ1:HI33(at CEN4) | This study               |
| KAY539, 568  | MATα his3Δ1200 ura3Δ10 cen3ΔPGAL1-CEN3 ura3Δ1:HI33(at CEN6) | This study               |
| KAY591, 628  | MATα his3Δ1200 leu2Δ10 ura3Δ10 cen6ΔPGAL1-CEN3 ura3Δ1:HI33(at CEN6) | This study               |
| KAY579       | MATα his3Δ1200 ura3Δ10 ade2Δ1 cen6ΔPGAL1-CEN3 ura3Δ1:HI33(at CEN6) | This study               |
| KAY626       | MATα/ΔMATα his3Δ1200/Δhis3Δ1200 LEU2/leu2Δ10 ura3Δ10/ura3Δ10 ade2Δ1/ΔADE2 cen6ΔPGAL1-CEN3/ CEN6 ura3Δ1:HI33(at CEN6) | This study               |
| KAY625       | MATα/ΔMATα his3Δ1200/Δhis3Δ1200 LEU2/leu2Δ10 ura3Δ10/ura3Δ10 ade2Δ1/ΔADE2 cen6ΔPGAL1-CEN3/ CEN6 ura3Δ1:HI33(at CEN6) | This study               |
the primers URA3_del_F and URA3_del_R, which flank the URA3 coding region.

KAY539 and KAY568, which carry a modified chromosome VI, were constructed with the same methods as were KAY614 and KAY619, except the primers CEN6_REPL_F and CEN6_REPL_R were used to target P_{GAL1}-CEN3 URA3 to replace CEN6, and the primer CEN6_F was used with URA3_int_R in a PCR to confirm that the conditional centromere had integrated at CEN6. KAY591 and KAY628 were constructed by crossing KAY539 and KAY568, respectively, to KAY519.

**Induction and selection of N+1 disomes**

Haploid strains carrying P_{GAL1}-CEN3 ura3::HIS3 at the centromere of the target chromosome were grown to saturation (2 days) in supplemented minimal medium that contained raffinose as its nonrepressing carbon source [40], diluted at least 2000-fold into fresh medium and grown overnight to obtain log-phase cultures. The cultures were split when the density was 0.5–1 × 10^7 cells/ml. To one half, galactose was added to a final concentration of 1.5%. Cell density was monitored until cultures had grown approximately 1.3 doublings (2.5-fold increase in density). The cells were pelleted, resuspended in water, diluted and plated to YPD and selective media lacking uracil or lacking both histidine and uracil. Ura^+ colonies were scored to determine frequency of duplication and excision, and were picked as candidate disomes (direct selection method). Colonies that grew on YPD were scored to determine viable cell density of plated cultures, and replica-plated to selective plates lacking uracil (to monitor excision) and to plates lacking histidine and uracil (to monitor duplication and excision). Replica-plated colonies on which many Ura^+His^+ papillae grew were considered candidate disomes and Ura^+His^+ papillae were picked (delayed selection method).

**Microarray-based comparative genomic hybridization (array CGH)**

Microarrays were produced by spotting PCR-amplified DNA fragments from approximately 6200 yeast open reading frames (kindly donated by D. Botstein, Princeton University) onto poly-lysine coated glass slides as described [41]. Genomic DNA was isolated by glass bead lysis according to the protocol of Hoffman and Winston [42]. To label each sample, 2 μg DNA was digested with HaeIII, purified, then resuspended in water. The DNA was boiled in the presence of 15 μg random nonamer nucleotide primers, then cooled on ice. The hybridized primers were extended with the use of 20 units of exon Klenow polymerase (New England Biolabs) in a 50 μl reaction containing 180 μM each of dATP, dGTP, dCTP, 72 μM dTTP, 108 μM 5-(3-aminoallyl)-dUTP, 50 mM NaCl, 10 mM Tris (pH 7.9), 10 mM MgCl_2, and 1 mM dithiothreitol. After 2 hours at 37°C, EDTA (pH 8.0) was added to 45 mM. The primer-extension products were purified through a DNA Clean and Concentrator-5 spin column (Zymo Research) and resuspended in 50 mM sodium bicarbonate (pH 9). Reactive Cy3 (or Cy5) mono NHS ester dyes (GE Healthcare, Cat. No. PN5661) were coupled to the aminoallyl groups in the DNA as directed by the supplier. The labeled DNA was purified through another spin column and resuspended in 20 μl 10 mM Tris (pH 8.5). Cy3- and Cy5-labeled DNAs were combined and hybridized to the microarrays at 65°C for 18 hours in a solution of 3× SSC, 730 μg/ml PolyA RNA, 240 μg/ml tRNA, 24 mM HEPES buffer (pH 7), and 0.24% SDS. Arrays were washed in 0.05× SSC at room temperature and imaged with a GenePix 4000B scanner (Molecular Devices). Array images were analyzed with ScanAlyze [43]. Data was filtered for signal intensity at least 2-fold above background in both channels, ratios were normalized to average 1 across all unaffected chromosomes, and log ratios were visualized with the Karyoscope viewer of Java Treeview [44].

**Accession number**

The raw microarray data, accession number GSE14377, are deposited at GEO [45].

**List of abbreviations**

CGH: comparative genomic hybridization; PCR: polymerase chain reaction; YPD: yeast extract, peptone, and dextrose; YP-galactose: yeast extract, peptone, and galactose

**Authors’ contributions**

KA conceived and designed the experiments, constructed yeast strains, performed yeast genetics and microarray experiments, and wrote the paper. JK constructed yeast strains, performed yeast genetics and microarray experiments, and drafted an early version of the paper. KK constructed yeast strains, performed yeast genetics and microarray experiments. IS constructed a plasmid, and performed yeast genetics experiments. EM and CS performed yeast genetics experiments. DP constructed yeast strains and performed yeast genetics experiments. All authors read and approved the final manuscript.
Additional material

Additional file 1
Characterization of conditional centromere. (A) Conditional centromere does not cause growth defects on glucose. The heterozygous parent of KAY614, containing pCAL2-CEN3 and ura3::HIS3 at the CEN4 locus, was sporulated and tetrads were dissected onto YPD rich medium. (B) Kinetics of galactose-induced chromosome loss. A diploid strain, heterozygous for pCAL2-CEN3 URA3 at CEN3, was grown in YPD to log phase, washed and incubated in YP-galactose, plated to YPD, then phenotyped. The non-repressing sugar raffinose was used in later experiments instead of glucose [40], which is expected to allow more rapid induction of GAL1 promoter activity. (C) Galactose-induced loss of chromosome IV yields unstable 2N-1 phenotype. A diploid strain, heterozygous for pCAL2-CEN3 URA3 at CEN4, was grown overnight in YPD or YP-galactose, then plated to YPD. Most of the small colonies were Ura- and unstable, rapidly reverting to normal growth but remaining Ura+. This is consistent with endoreduplication of the remaining chromosome IV, as observed by Alturo et al. [29].

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Additional file 2
Galactose induction of candidate chromosome VI disomes. Galactose induces the appearance of many small Ura-His' papillae in a strain carrying a modified chromosome VI. Strain KAY628, harbouring the LEU2-marked TUB1 plasmid pKAS5, was grown to log phase in raffinose-containing medium, split, and one-half was exposed to galactose for 1.3 cultural doublings. 104 cells were spread to plates selecting for Ura-His-Leu' papillae. Plates were incubated 3 days and photographed. Papillae were picked, colony-purified, then cultured for DNA isolation and array CGH as described in the text.

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Additional file 3
Oligonucleotides used in this study. This table contains nucleotide sequences and genome coordinates of the oligonucleotides used for PCR.

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Additional file 4
Strain construction summaries. This file contains flowcharts that summarize the construction of yeast strains in this study.

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References
1. Torres EM, Williams BR, Amon A: Aneuploidy: cells losing their balance. Genetics 2008, 179(2):737-746.
2. Birchler JA, Veitia RA: The gene balance hypothesis: from classical genetics to modern genomics. Plant Cell 2007, 19(2):395-402.
3. Torres EM, Sokolsky T, Tucker CM, Chan LY, Boselli M, Dunham MJ, Amon A: Effects of aneuploidy on cellular physiology and cell division in haploid yeast. PLoS Genet 2006, 2(7):e111.
4. Cox BS, Bevan EA: Aneuploidy in yeast. New Phytologist 1962, 61(3):342-355.
5. Hughes TR, Roberts CJ, Dai H, Jones AR, Meyer MR, Slade D, Burghard J, Dow S, Ward TR, Kidd MJ, et al: Widespread aneuploidy revealed by DNA microarray expression profiling. Nat Genet 2000, 25(3):333-337.
6. Campbell D, Doctor JS, Feuersanger JH, Doolittle MM: Differential mitotic stability of yeast disomes derived from triploid meiosis. Genetics 1981, 98(2):239-255.
7. Shaffer B, Brearley I, Littlewood R, Fink GR: A stable aneuploid of Saccharomyces cerevisiae. Genetics 1971, 71(4):483-495.
8. Conde J, Fink GR: A mutant of Saccharomyces cerevisiae defective for nuclear fusion. Proc Natl Acad Sci USA 1976, 73(10):3651-3653.
9. Nilsson-Tillgren T, Petersen JGL, Holmberg S, Kielland-Brandt MC: Transfer of chromosome III during kary mediated cytoduction in yeast. Carlsberg Research Communications 1980, 45(2):113-117.
10. Dutcher SJ: Internuclear transfer of genetic information in kar1-kAR1 heterokaryons in Saccharomyces cerevisiae. Mol Cell Biol 1981, 1(3):245-253.
11. Rieder CL, Maito H: Stuck in division or passing through: what happens when cells cannot satisfy the spindle assembly checkpoint. Dev Cell 2004, 7(5):673-683.
12. Biggins S, Walczak CE: Captivating capture: how microtubules attach to kinetochores. Curr Biol 2003, 13(11):R449-460.
13. Hill A, Bloom K: Genetic manipulation of centromere function. Mol Cell Biol 1987, 7(7):2397-2405.
14. Chan CS, Botstein D: Isolation and characterization of chromosome gain and increase-in-ploidy mutants in yeast. Genetics 1993, 135(3):677-691.
15. Baudin A, Ozier-Kalogeropoulos O, Denouel A, Lacroute F, Cullin C: Internuclear transfer and genetic information in heterokaryons in Saccharomyces cerevisiae. Mol Cell 1989, 9(3):1368-1370.
16. Ossowska KA, Castillo AR, Tatsutani SY, Biggins S: De novo kinetochore assembly requires the centromeric histone H3 variant. Mol Biol Cell 2005, 16(12):5649-5660.
17. Schiestl RH, Igarashi S, Hastings PJ: Analysis of the mechanism for reversion of a disrupted gene. Genetics 1988, 119(2):237-247.
18. Cherry JM, Ball C, Weng S, Juvik G, Schmidt R, Adler C, Dunn B, Dwight S, Riles L, Mortimer RK, et al: Genetic and physical maps of Saccharomyces cerevisiae. Nature 1997, 387(6632 Suppl):S7-73.
19. Hunter N, Chambers SR, Louis EJ, Borts RH: The mismatch repair system contributes to meiotic sterility in an interspecific yeast hybrid. Embo J 1996, 15(7):1726-1733.
20. Liu H, Krieger J, Breitcaster A: Construction of a GAL1-regulated yeast cDNA expression library and its application to the identification of genes whose overexpression causes lethality in yeast. Genetics 1992, 132(3):665-673.
21. Moriya H, Shimizu-Yoshida Y, Kitano H: In vivo robustness analysis of cell division cycle genes in Saccharomyces cerevisiae. PLoS Genet 2006, 2(7):e111.
24. Vinh DB, Drubin DG: A yeast TCP-1-like protein is required for actin function in vivo. Proc Natl Acad Sci USA 1994, 91(19):9116-9120.
25. Vlasin A, Craig K, Hwang E, Prinz S, Tyers M, Amon A: The phosphatase Cdc14 triggers mitotic exit by reversal of Cdk-dependent phosphorylation. Mol Cell 1998, 2(6):709-718.
26. Schatz P, Solomon F, Botstein D: Genetically essential and non-essential alpha-tubulin genes specify functionally interchangeable proteins. Mol Cell Biol 1986, 6(11):3722-3733.
27. Weinstein B, Solomon F: Phenotypic consequences of tubulin overproduction in Saccharomyces cerevisiae: differences between alpha-tubulin and beta-tubulin. Mol Cell Biol 1990, 10(10):5295-5304.
28. Reid RJ, Sunjevaric I, Voth WP, Ciccone S, Du W, Olsen AE, Stillman DJ, Rothstein R: Chromosome-scale genetic mapping using a set of 16 conditionally stable Saccharomyces cerevisiae chromosomes. Genetics 2008, 180(4):1799-1808.
29. Visintin R, Craig K, Hwang E, Prinz S, Tyers M, Amon A: Chromosome-scale genetic mapping using a set of 16 conditionally stable Saccharomyces cerevisiae chromosomes. Yeast 2006, 23(14–15):1097-1106.
30. Amberg DC, Burke DJ, Stratmann JN: Methods in Yeast Genetics: a Cold Spring Harbor Laboratory course manual. 2005 edition. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press; 2005.
31. Kassir Y, Simchen G: Monitoring meiosis and sporulation in Saccharomyces cerevisiae. Methods Enzymol 1991, 194:94-110.
32. Sikorski RS, Hieter P: A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in Saccharomyces cerevisiae. Genetics 1989, 122(1):19-27.
33. Christianson TW, Sikorski RS, Dante M, Shero JH, Hieter P: Multi-functional yeast high-copy-number shuttle vectors. Gene 1992, 110(1):119-122.
34. Rozen S, Skaletsky H: Primer3 on the WWW for general users and for biologist programmers. Methods Mol Biol 2000, 132:365-386.
35. Sambrook J, Russell DW: Molecular Cloning: A Laboratory Manual. 3rd edition. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press; 2001.
36. Ma H, Kunes S, Schatz PJ, Botstein D: Plasmid construction by homologous recombination in yeast. Gene 1987, 58(2-3):201-216.
37. Winston F, Dollard C, Ricupero-Hovasse SL: Construction of a set of convenient Saccharomyces cerevisiae strains that are isogenic to S288C. Yeast 1995, 11(1):53-55.
38. Brachmann CB, Davies A, Cost GJ, Caputo E, Li J, Hieter P, Boeke JD: Designer deletion strains derived from Saccharomyces cerevisiae S288C: a useful set of strains and plasmids for PCR-mediated gene disruption and other applications. Yeast 1998, 14(2):115-132.
39. Rose M, Winston F: Identification of a Ty insertion within the coding sequence of the S. cerevisiae URA3 gene. Mol Gen Genet 1984, 193(3):557-560.
40. Johnston M, Flick JS, Pexton T: Multiple mechanisms provide rapid and stringent glucose repression of GAL gene expression in Saccharomyces cerevisiae. Mol Cell Biol 1994, 14(6):3834-3841.
41. DeRisi JL, Iyer VR, Brown PO: Exploring the metabolic and genetic control of gene expression on a genomic scale. Science 1997, 278(5338):680-686.
42. Hoffman CS, Winston F: A ten-minute DNA preparation from yeast efficiently releases autonomous plasmids for transformation of Escherichia coli. Gene 1987, 57(2-3):267-272.
43. ScanAlyze [http://rana.lbl.gov/EisenSoftware.htm].
44. Saldanha AJ: Java Treeview – extensible visualization of microarray data. Bioinformatics 2004, 20(17):3246-3248.
45. Gene Expression Omnibus [http://www.ncbi.nlm.nih.gov/geo/].