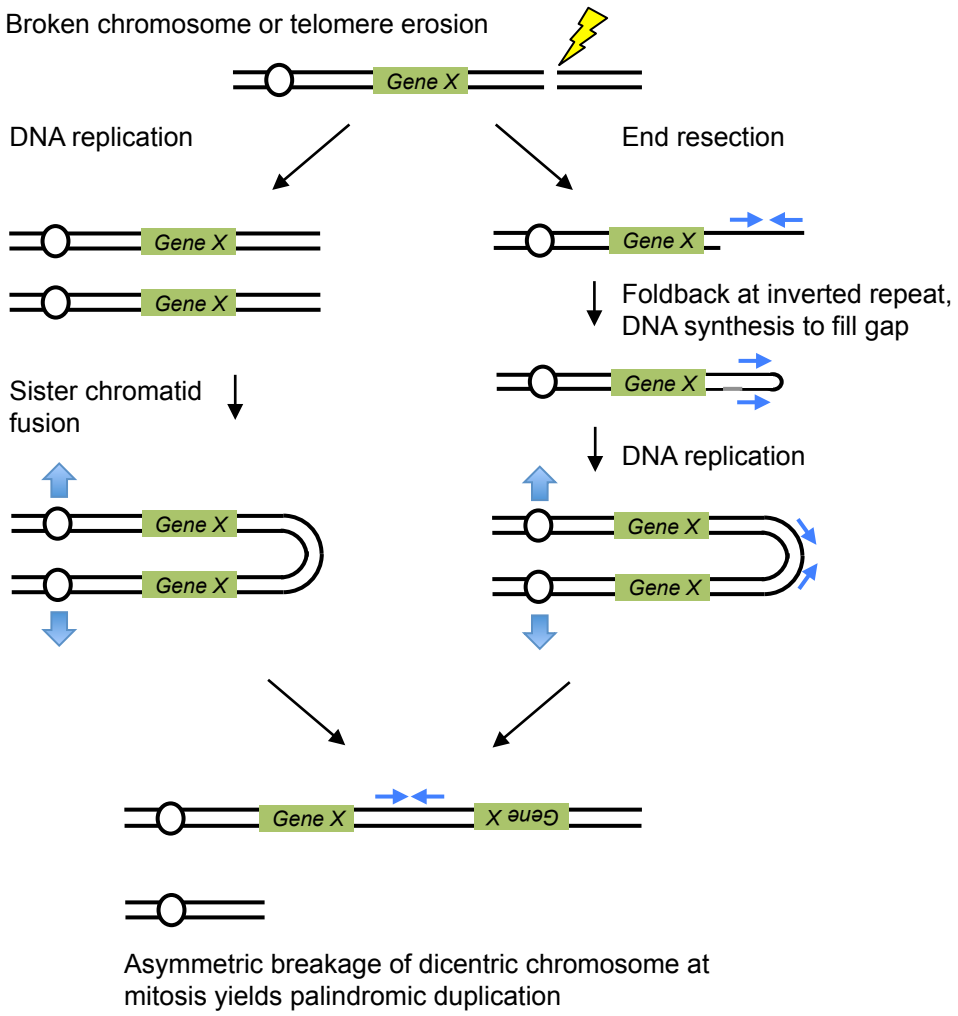


**Figure S1, related to Figures 1 and 3**. Models for palindromic duplication by break-fusion-bridge cycles.

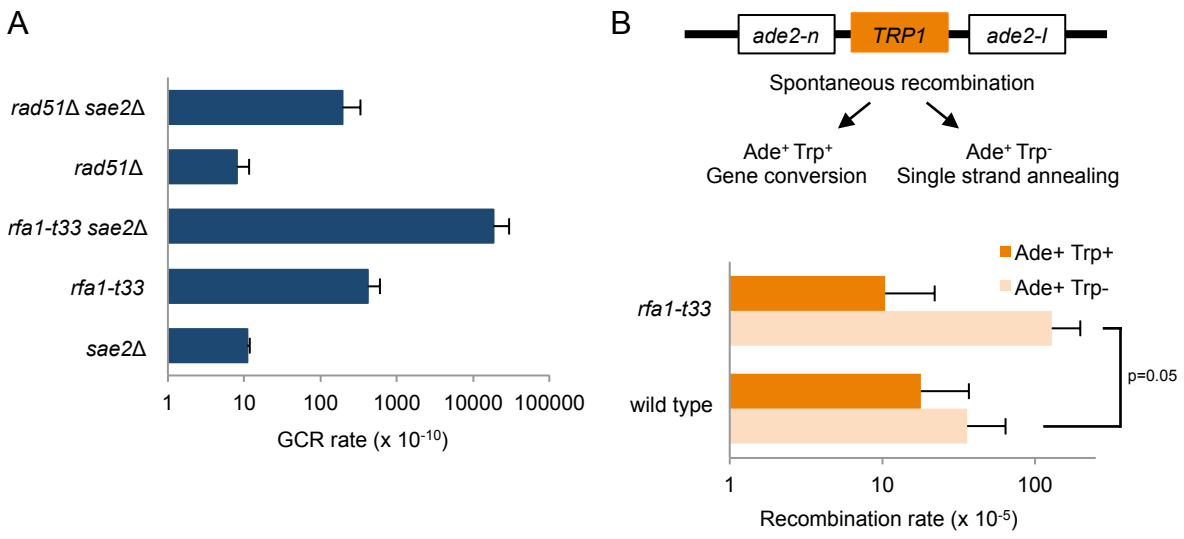


**Figure S2, related to Figure 1.** Increased GCR rate in the *rfa1-t33* mutant is not due to a recombination defect.
(A) GCR rates for the indicated genotypes. The rates shown for *rad51*Δ and *rad51*Δ *sae2*Δ are the average of three and four independent trials, respectively. Error bars indicate standard deviation.
(B) Recombination rates for wild type and *rfa1-t33* strains. The rates shown are the average of three independent trials. Error bars indicate standard deviation.

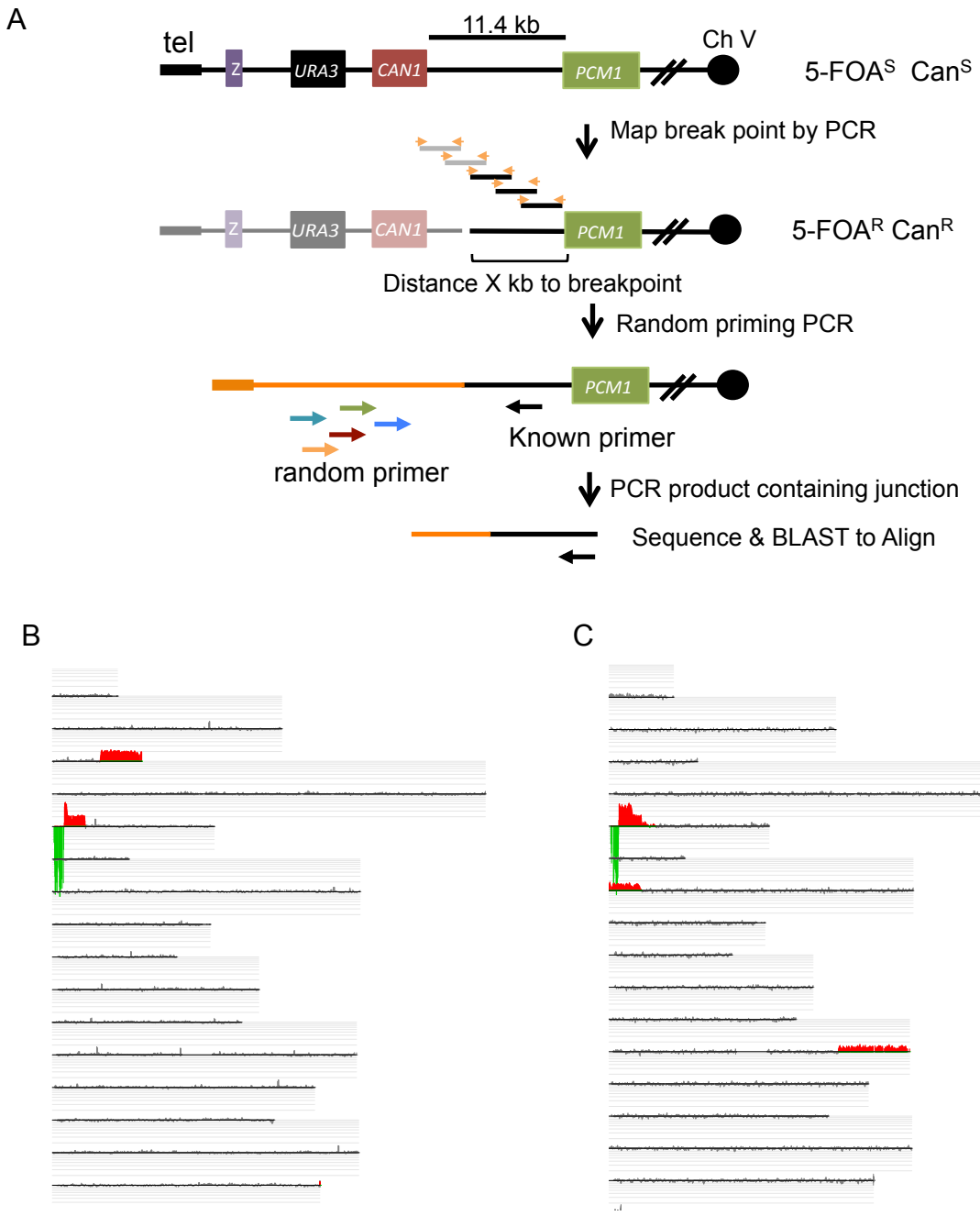


**Figure S3, related to Figure 2 and Table 1.** Schematic of the PCR assay used to characterize GCRs and CGH of clones with >2-fold amplification of Ch V sequences.
(A) Overlap PCR defines the breakpoint between *CAN1* and *PCM1* then random priming PCR was used to amplify the junction. PCR fragments were sequenced and aligned to the reference genome to define the sequences involved in the rearrangements.
(B) A 10-kb region adjacent to the breakpoint is present in 4 copies.
(C) A 4-fold amplification of a 48 kb region adjacent to the breakpoint. The clone shown represents a mixed population of two clones with the same rearrangement on Ch V associated with distinct secondary rearrangements involving Ch VII and Ch XII (determined by clonal analysis).

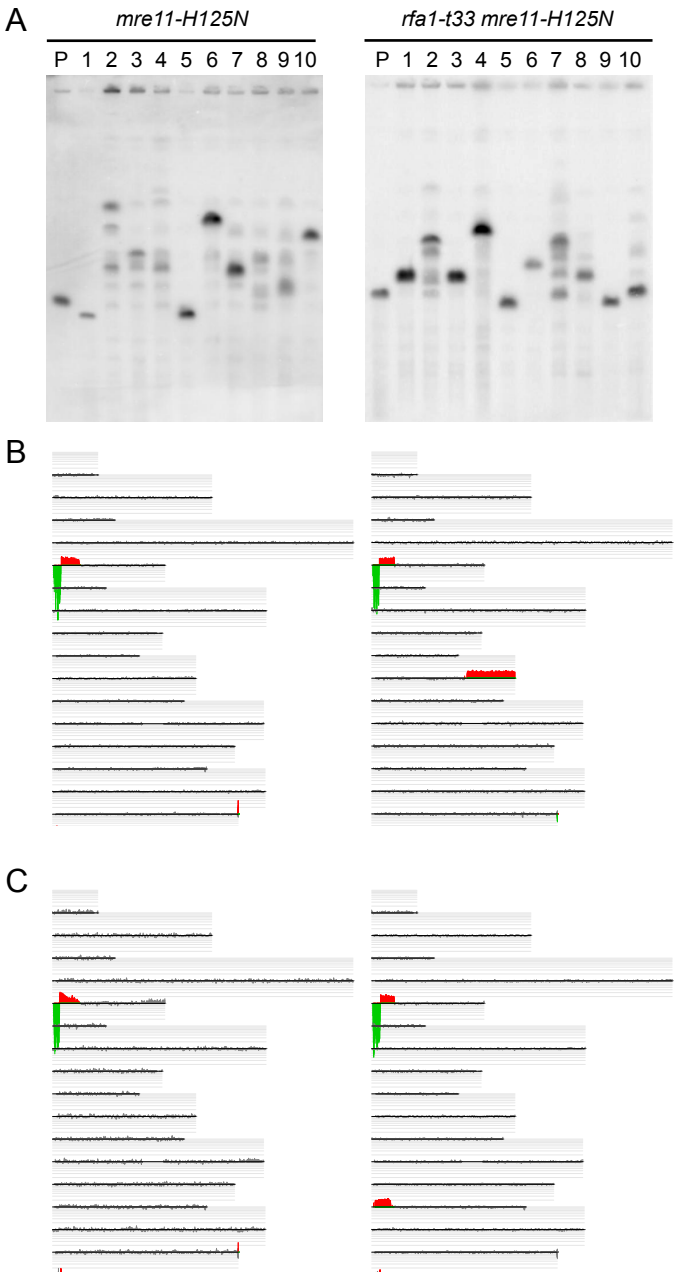


**Figure S4, related to Figures 1 and 3.** Inversion duplications are recovered from *mre11-H125N* derivatives.
(A) PFGE of independent GCR clones.
(B) Representative microarrays from *mre11-H125N* clones.
(C) Representative microarrays from and *rfa1-t33 mre11-H125N* clones.

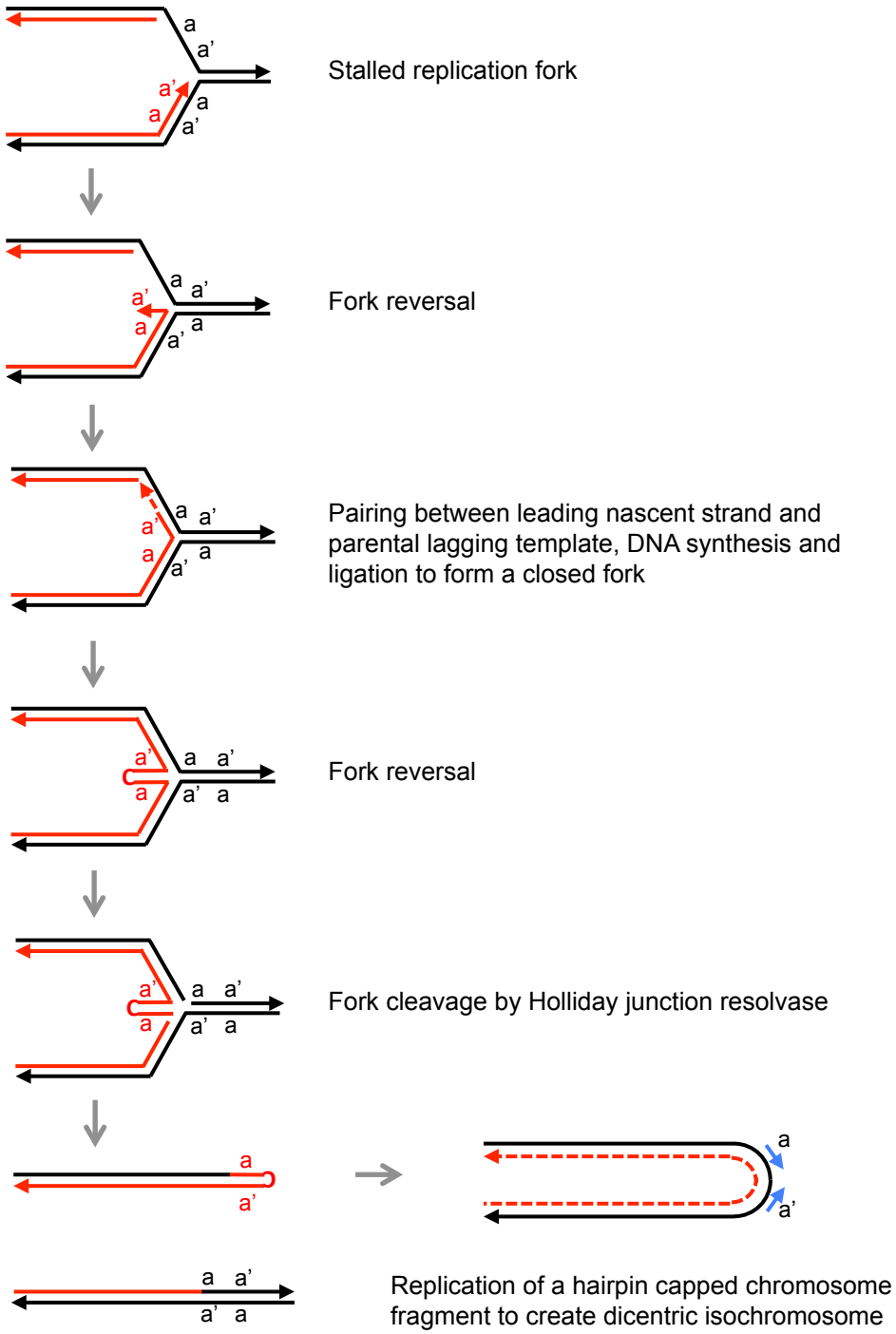


**Figure S5, related to Figure 3.** Model for generation of a dicentric chromosome by template switching between inverted repeats at a stalled replication fork. a and a' represent short inverted repeats

**Table S1, related to Figure 3. Inverted repeats initiate inverted duplications**

| Relevant genotype | Inverted Repeat Sequence[1] | No. of Events | Ch V coordinate |
|---|---|---|---|
| *sae2*Δ | CCCAGG**ca**CCTGGG | 1 | 32,659 |
| | TATATtTCTG**ttc**CAGAtATATA | 1 | 33,588 |
| | GAGTTT**ctca**AAACTC | 1 | 35,581 |
| | TAA - GCCAC**tgca**GTGGCaTTA | 1 | 42,134 |
| | CGCCA**ctcccgcagtcc**TGGCG | 1 | 42,109 |
| *sae2*Δ *rfa1-t33* | TTCcaGGGCAA**aagtga**TTGCCCaaGAA | 4 | 32,915 |
| | CACTT**gccagt**AAGTG | 1 | 34,006 |
| | CTCgTGGG**cgct**CCCAtGAG | 1 | 41,663 |
| | TATATtTCTG**ttc**CAGAtATATA | 1 | 33,588 |

[1] Bases in lower case represent mismatches within the inverted repeat, and bases in bold lower case indicate the spacer between inverted repeats.

**Supplemental Experimental Procedures**

**Media, growth conditions and yeast strains.** Rich medium (yeast extract-peptone-dextrose, YPD) and synthetic medium (SC) were as described previously (Amberg, 2005). Selection for GCR events was performed using synthetic complete medium without arginine containing 1mg/mL 5-fluoroorotic acid (5-FOA) and 60µg/mL cananvanine (Can) as described (Chen and Kolodner, 1999). Yeast strains are derivatives of RDKY3615 (*MAT***a** *ura3-52 leu2*Δ*1 trp1*Δ*63 his3*Δ*200 lys2*Δ*BglI hom-10 ade2*Δ*1 ade8*) (Chen and Kolodner, 1999). The *sae2*Δ deletion strains, LSY2706 and LSY2707, were generated by one-step gene-replacement of RDKY3615 and RDKY3617, respectively with *sae2::KanMX* PCR products. The *mre11-H125N* derivatives, LSY3388 and LSY3389, were created by one-step replacement of RDKY3615 and RDKY3617 with *mre11-H125N-NatMX* PCR products. The *rad51*Δ (LSY3441) and *rad51*Δ *sae2*Δ (LSY3442) strains were generated by one-step gene replacement of RDKY3615 and LSY2706 with the XbaI/PstI fragment from the pAM28 plasmid (Rattray and Symington, 1994). Wild type (LSY3449-18A) and *rfa1-t33* (LSY3449-10D) strains bearing the *ade2* direct repeat were generated by crossing appropriate haploids (Mozlin et al., 2008).

**GCR assays and PCR mapping**. Fluctuation assays to determine the rate of GCRs, PCR mapping and amplification of the junctions were performed as previously described (Putnam and Kolodner, 2010);(Schmidt et al., 2006). Some telomere addition events were identified using a terminal transferase-mediated PCR method (Forstemann et al., 2000). Briefly, terminal transferase (New England Biolabs) was used to add a C-tail to DNA ends. Then, a 5'-(CGGGATCC)$G_{18}$-3' primer and primer that anneals only adjacent to the breakpoint was used to PCR amplify the region and sequenced. For physical analysis of inversion duplications, 3 µg of genomic DNA was digested with 20 units of the indicated restriction endonucleases, separated by gel electrophoresis and transferred to Biobond-Plus nylon membrane (Sigma) for hybridization.

**PFGE and aCGH**. Samples for PFGE were obtained from 7 mL saturated yeast cultures in YPD. Cells were embedded in low-melt agarose and lysed as previously described (Amberg, 2005). Chromosomes were separated by CHEF-DR II Pulsed-Field Electrophoresis system (BioRad) following a published protocol (Argueso et al., 2008). Chromosomes were transferred to nylon membranes and hybridized with a radiolabeled *PCM1* probe to identify Ch V rearrangements. Agarose plugs were melted and the DNA was sonicated, extracted and labeled for microarray hybridization as previously described (Zhang et al., 2013).

**Bisulfite sequencing**

2 µg of genomic DNA was treated with the EpiMark Bisulfite Conversion kit (New England Biolabs) according to the manufacturers instructions. Bisulfite treated DNA was used for PCR amplification using EpiMark Hot Start Taq DNA Polymerase (New England Biolabs) according to the manufacturers instructions.

**Determination of spontaneous mitotic recombination rates:** Mitotic recombination rates between *ade2* direct repeats were determined as described previously (Mozlin et al., 2008).

**References**

Amberg, D.C., Burke, D. J. & Strathern, J. N. (2005). Methods in Yeast Genetics: A Cold Spring Harbor Laboratory Course Manual. Cold spring Harbor Laboratory Press.

Argueso, J.L., Westmoreland, J., Mieczkowski, P.A., Gawel, M., Petes, T.D., and Resnick, M.A. (2008). Double-strand breaks associated with repetitive DNA can reshape the genome. Proceedings of the National Academy of Sciences of the United States of America *105*, 11845-11850.

Chen, C., and Kolodner, R.D. (1999). Gross chromosomal rearrangements in Saccharomyces cerevisiae replication and recombination defective mutants. Nat Genet *23*, 81-85.

Forstemann, K., Hoss, M., and Lingner, J. (2000). Telomerase-dependent repeat divergence at the 3' ends of yeast telomeres. Nucleic acids research *28*, 2690-2694.

Mozlin, A.M., Fung, C.W., and Symington, L.S. (2008). Role of the Saccharomyces cerevisiae Rad51 paralogs in sister chromatid recombination. Genetics *178*, 113-126.

Putnam, C.D., and Kolodner, R.D. (2010). Determination of gross chromosomal rearrangement rates. Cold Spring Harb Protoc *2010*, pdb prot5492.

Rattray, A.J., and Symington, L.S. (1994). Use of a chromosomal inverted repeat to demonstrate that the RAD51 and RAD52 genes of Saccharomyces cerevisiae have different roles in mitotic recombination. Genetics *138*, 587-595.

Schmidt, K.H., Pennaneach, V., Putnam, C.D., and Kolodner, R.D. (2006). Analysis of gross-chromosomal rearrangements in Saccharomyces cerevisiae. Methods Enzymol *409*, 462-476.

Zhang, H., Zeidler, A.F., Song, W., Puccia, C.M., Malc, E., Greenwell, P.W., Mieczkowski, P.A., Petes, T.D., and Argueso, J.L. (2013). Gene copy-number variation in haploid and diploid strains of the yeast Saccharomyces cerevisiae. Genetics *193*, 785-801.