Fig.S1

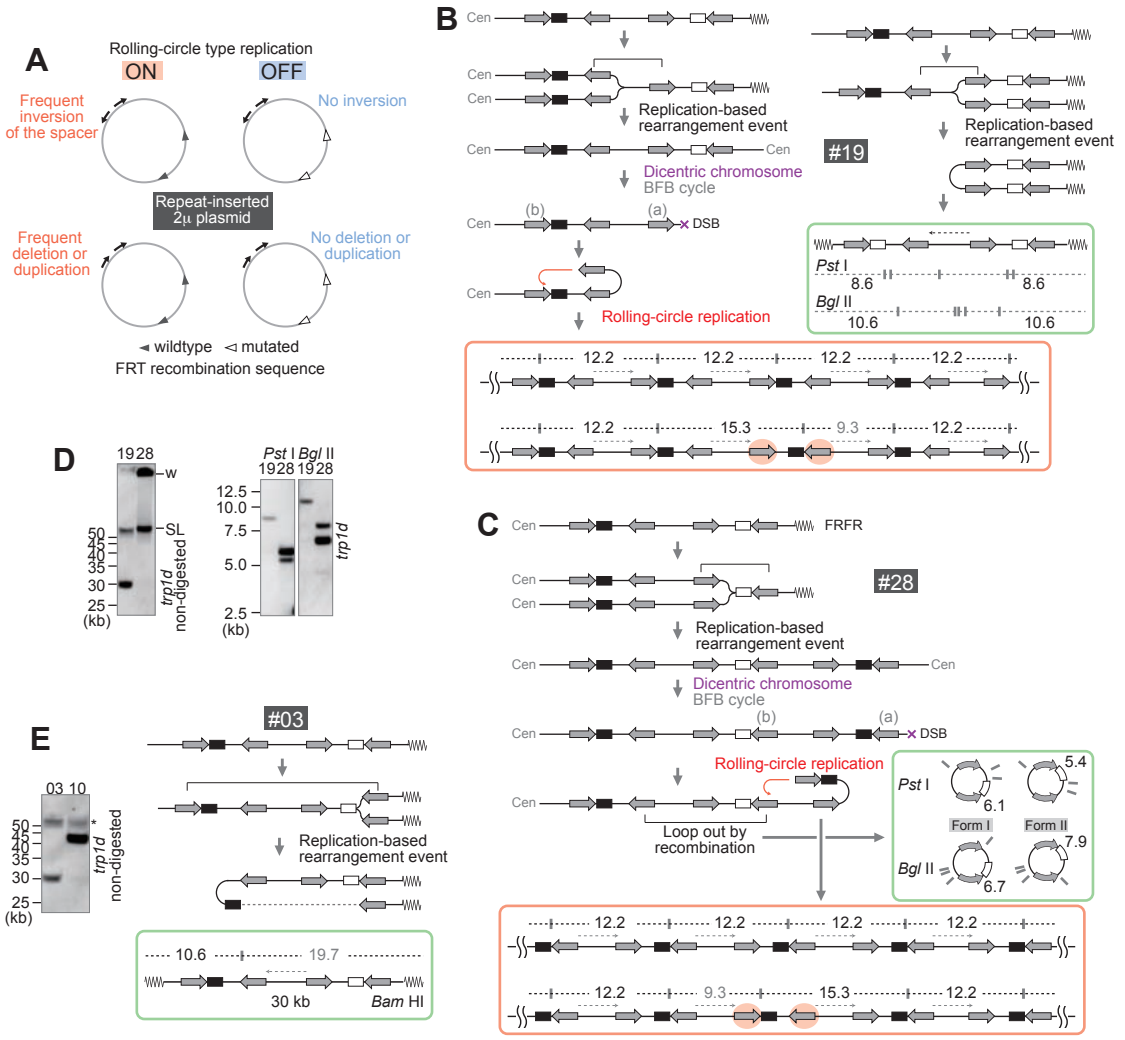

Fig. S1 Characterization of complex events of amplification.
(**A**) Direct association between rolling-circle type replication (RCR) and intensive rearrangements. The yeast 2μ plasmid encodes a site-specific recombinase, Flp1p, and contains a pair of Flp1p recombinase target (FRT) sites. The Flp1-FRT recombination can initiate RCR. We previously showed frequent rearrangements at repeats inserted in the RCR-proficient plasmid, but not in the RCR-deficient plasmid with mutated FRT sites.
(**B**) Predicted processes for the FRFR #19 colony. The replication-based rearrangement event forms a 30-kb extra-chromosome that contains the trp1d marker only (right). The PstI and BglII maps agree well with the result of the Southern analysis in (C). Another subsequent event produced intra-chromosomal amplification through dicentric chromosome formation (left). The RCR-amplification produced original 12.2-kb fragments and rearranged 15.3-kb fragments that contain the *leu2d* marker only. The BamHI-map indicates a leu2d-containing fragment (black) and a non-hybridizing fragment (gray).
(**C**) Predicted processes for the FRFR #28 colony. The replication-based rearrangement event forms a dicentric chromosome that causes RCR-amplification and looping out of a circular amplicon. The circular products contain two structural variants, and their PstI and BglII maps agree well with the result of the Southern analysis in (C). The BamHI-map indicates a leu2d-containing fragment (black) and a non-hybridizing fragment (gray).
(**D**) Southern blots for analyzing complex amplification events with the *trp1d* probe. (Left) The samples, FRFR #19 and 28 in Fig. 1, B-D, were analyzed using field-inversion gel electrophoresis. W: well position; SL: separation limit. (Right) PstI or BglII digested samples.
(**E**) (Left) Southern blot of extra-chromosomal DNA with the *trp1d* probe. The samples, FRFR #03 and 10 in Fig. 1, B-D, were analyzed using field-inversion gel electrophoresis. Black asterisks on the right side of panels indicate separation limit under the PFGE-condition. (Right) A predicted process for 30-kb extra-chromosomal amplification. The replication-based rearrangement event between IRs marked with a bracket forms a 30-kb extra-chromosome, as shown in Fig. 3D. The *Bam*HI-map indicates a leu2d-containing fragment (black) and a non-hybridizing fragment (gray).

Fig.S2

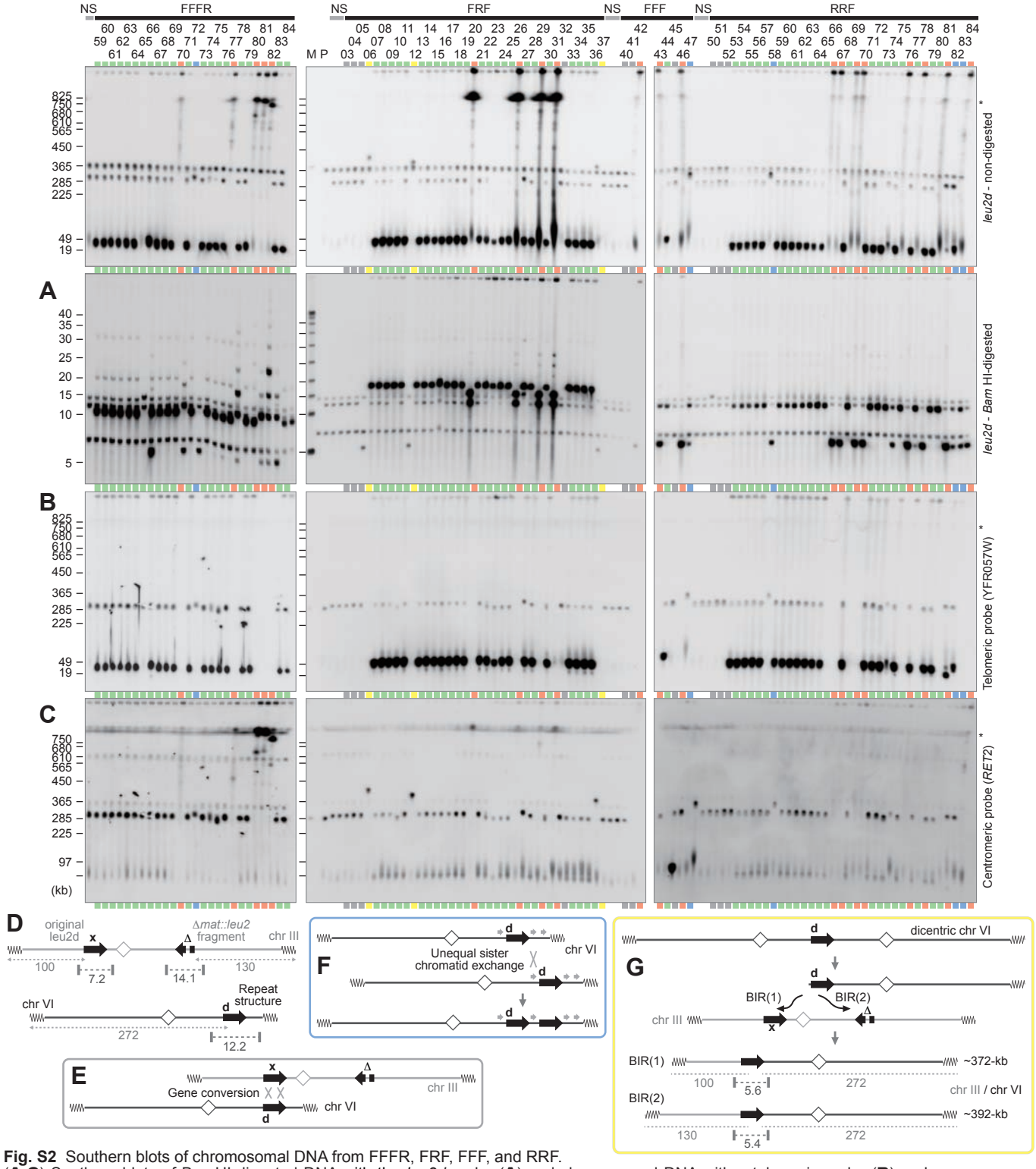

Fig. S2 Southern blots of chromosomal DNA from FFFR, FRF, FFF, and RRF.
(**A**-**C**) Southern blots of BamHI-digested DNA with the *leu2d* probe (**A**) and chromosomal DNA with a telomeric probe (**B**) and a centromeric probe (**C**). The samples marked in red and green indicate intra- and extra-chromosomal products, respectively. The gray lane showed no sign of amplification, suggesting Leu+ conversion between the *leu2d* marker and the mutated original *leu2* allele on chr III. The blue samples suggest moderate copy number increase of the *leu2d* gene likely through unequal sister chromatid exchange. The yellow samples possibly contain a fusion between chromosome VI and III, which cause Leu+ recombination between the *leu2d* marker and the mutated original *leu2* allele on chr III. Black asterisks on the right side of panels indicate separation limit under the PFGE-condition. M: S. cerevisiae marker; P: the parental strain, LS20; NS: non-selective conditions.
(**D**) The structures of chromosomes III and VI of the parental strain, LS20.
The *leu2d* gene (black arrow with "d") within the constructed repeats produces a 12.2-kb BamHI fragment. The black arrows with "x" and "Δ" indicate the original leu2 allele and the partial*leu2* fragment for deletion of the MAT locus, respectively, which produce 7.2 and 14.1-kb *Bam*HI fragments, respectively. The segments forming a fusion chromosome III and VI (yellow lanes in A-C) are shown with double-headed dotted arrows.
(**E**-**G**) Predicted Leu+ processes in colonies in gray, blue, and yellow lanes.
(**E**) Gene conversion between the original *leu2* mutant allele on chromosome III and the leu2d gene within the constructed repeats.
(**F**) Unequal sister chromatid exchange duplicates (or further triplicates) the leu2d gene.
(**G**) Fusion of Chromosome III and VI. A broken end formed in a dicentric chromosome VI can interact with the original leu2 allele and undergoes a recombination-dependent DNA replication (break-induced replication, BIR), generating a ~372-kb fusion chromosome containing a LEU2 gene that produces a 5.6-kb *Bam*HI fragment (BIR1). Alternatively, the broken chromosome can invade into the partial leu2 fragment (with "Δ"), generating a ~392-kb fusion chromosome that produces a 5.4-kb *Bam*HI fragment (BIR2).

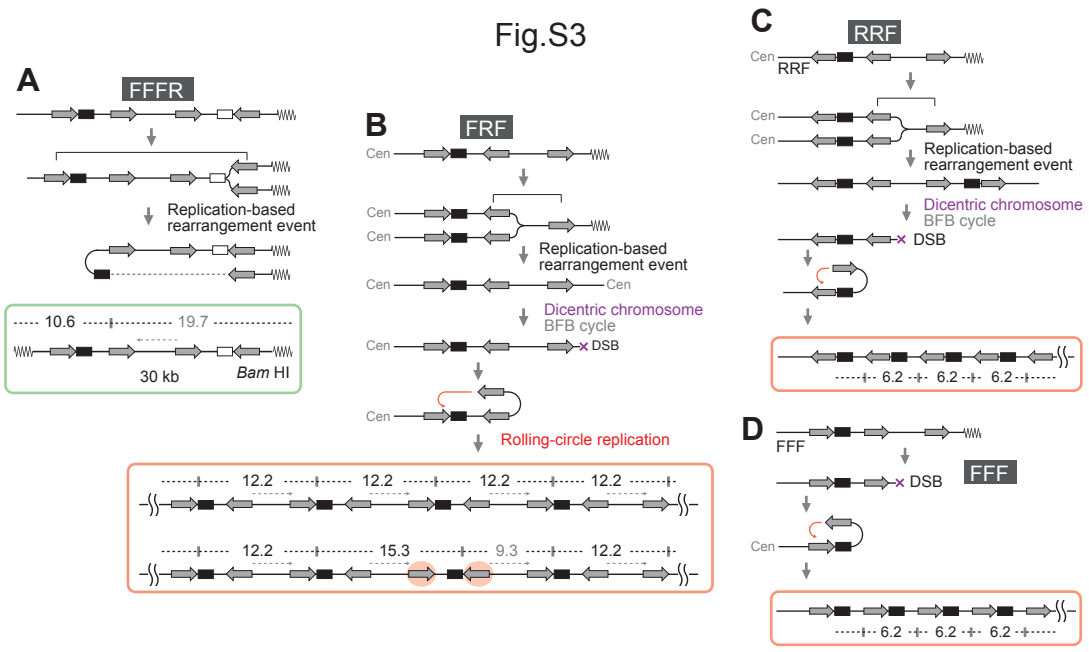

Fig. S3 Predicted processes for gene amplification from variants of repeat structure.
(**A**) A predicted process for FFFR extra-chromosomal amplification.
The replication-based rearrangement event between IRs marked with a bracket forms a 30-kb extra-chromosome, as shown in Fig. 3D. The BamHI-map indicates a leu2d-containing fragment (black) and a non-hybridizing fragment (gray).
(**B**) A predicted process for FRF intra-chromosomal amplification. The replication-based event produced intra-chromosomal amplification through dicentric chromosome formation. The RCR-amplification produced original 12.2-kb fragments and rearranged 15.3-kb fragments.
(**C**) A predicted process for RRF intra-chromosomal amplification. The replication-based event produced intra-chromosomal amplification through dicentric chromosome formation. The RCR-process formed simple tandem repeats, in which duplication or deletion does not affect the BamHI-fragment size (6.2-kb).
(**D**) A predicted process for FFF intra-chromosomal amplification. A spontaneous DSB by unidentified mechanism could cause RCR. The RCR-process formed simple tandem repeats, in which duplication or deletion does not affect the BamHI-fragment size (6.2-kb).

Fig.S4

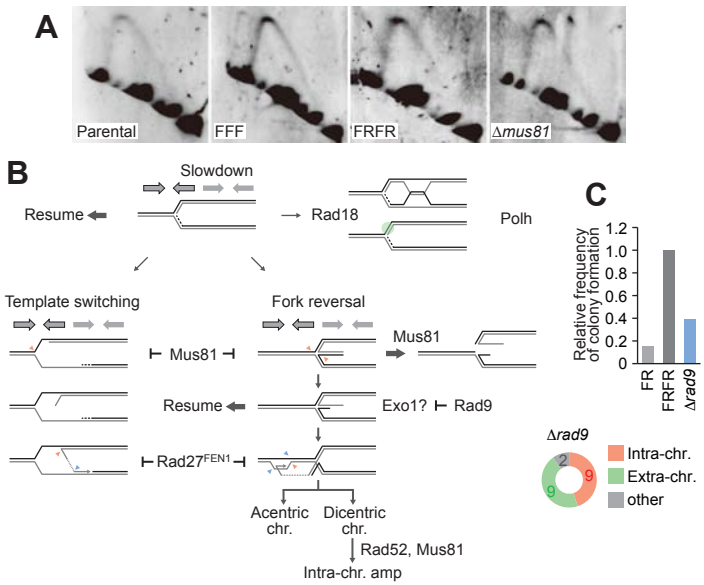

Fig. S4 A detailed picture of possible processes generating acentric/dicentric chromosomes.
(**A**) Repeat-associated replication stress assessed by 2D analysis. The analyzed region is shown in Fig. 5C. The dense signal at the top of the Y-arc could be occasionally observed in 2D assays, as a common feature. Please note the difference of large-Y intensity between samples. (**B**) A detailed model for processing slowdown forks within an IR-structure
Most slowdown forks would be resumed safely or rescued by post-replication repair pathways, such as hemicatenane formation or translesion DNA synthesis mediated by Rad18p (top). Slowdown forks may be uncoupled and accumulate single-strand DNA (ssDNA) gaps, which can suffer from faulty template switching (bottom left). In our system, however, chromosomal rearrangements occurred even through IRs that are 15.3-kb apart (Fig S3B and S5A), suggest-ing that other processes independent of such a long ssDNA gap would be favorable. Replica-tion fork slowdown could cause fork regression, which can be resolved by the Mus81 endonuclease (bottom right). The regressed forks could be resected by exonucleases and invade into an ectopic inverted sequence. The intermediates of ectopic invasion may be resolved by Rad27 (yeast FEN1). Steps linking dicentric chromosomes to intra-chromosomal amplification require a recombinational pathway involving Mus81 and Rad52.
(**C**) FRFR-amplification in rad9
(Top) Frequencies of colony formation in rad9. Means from two independent experiments were plotted. (Bottom) Types of amplification products in rad9.

Fig.S5

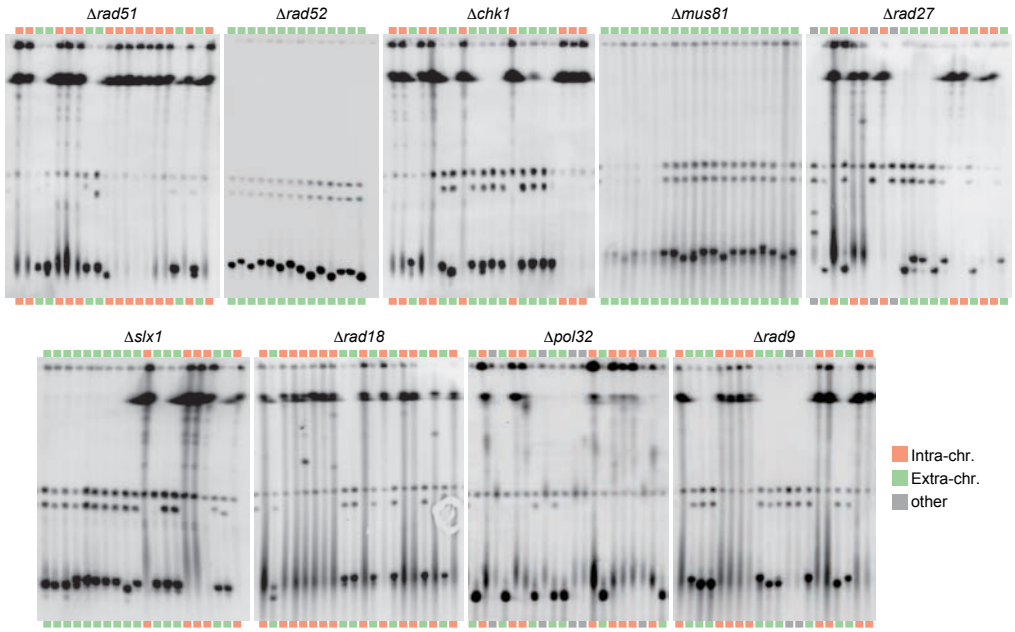

Fig. S5 PFGE-Southern images of FRFR-amplification in mutants.
Lanes with extra- or intra-chromosomal amplification are marked in green or red, respectively. Samples maked in gray indicate gene conversion, recombination, or only small copy number gains.

Fig.S6

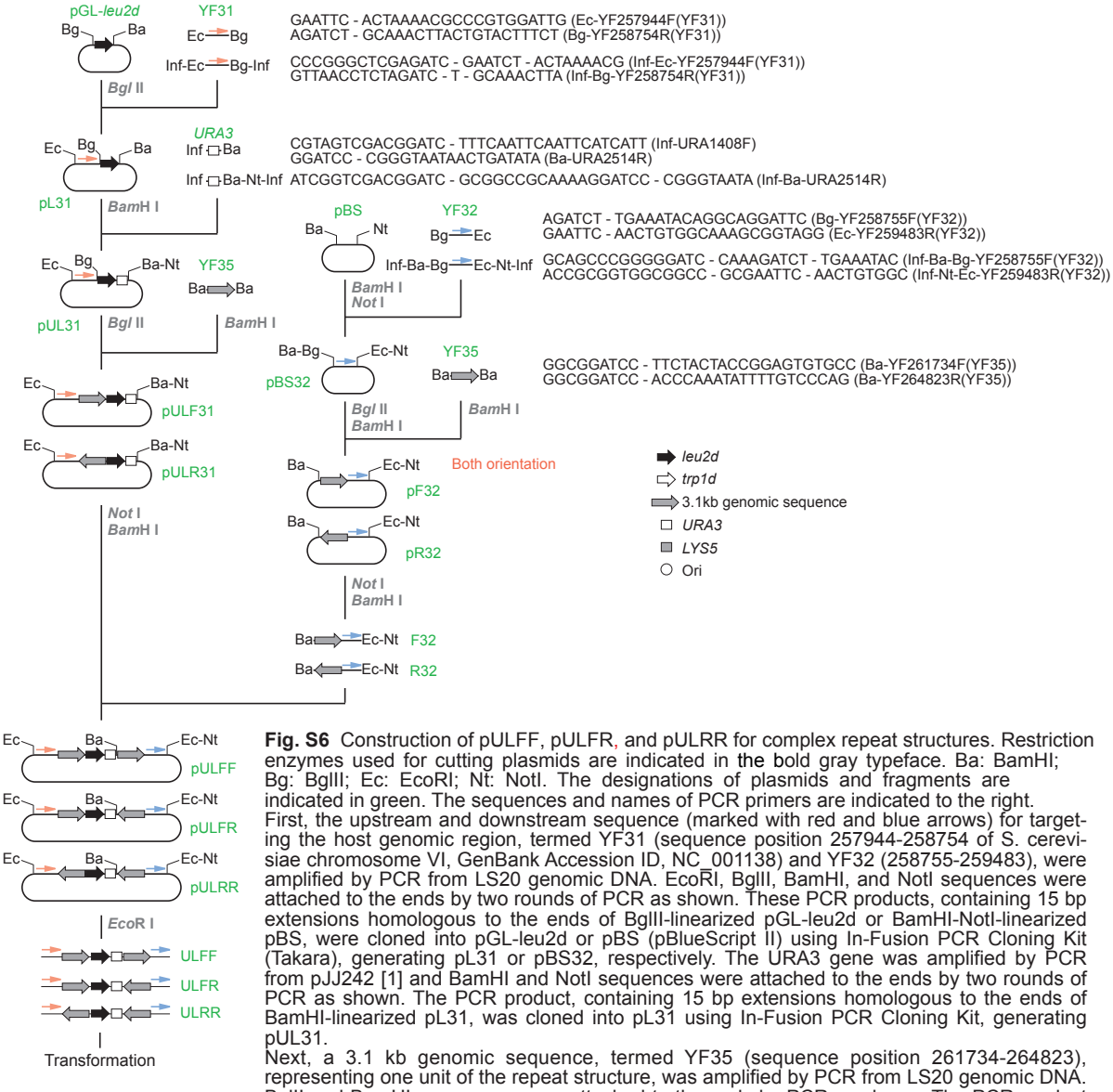

Fig. S6 Construction of pULFF, pULFR, and pULRR for complex repeat structures. Restriction enzymes used for cutting plasmids are indicated in the bold gray typeface. Ba: BamHI; Bg: BglII; Ec: EcoRI; Nt: NotI. The designations of plasmids and fragments are indicated in green. The sequences and names of PCR primers are indicated to the right.
First, the upstream and downstream sequence (marked with red and blue arrows) for targeting the host genomic region, termed YF31 (sequence position 257944-258754 of S. cerevisiae chromosome VI, GenBank Accession ID, NC_001138) and YF32 (258755-259483), were amplified by PCR from LS20 genomic DNA. EcoRI, BglII, BamHI, and NotI sequences were attached to the ends by two rounds of PCR as shown. These PCR products, containing 15 bp extensions homologous to the ends of BglII-linearized pGL-leu2d or BamHI-NotI-linearized pBS, were cloned into pGL-leu2d or pBS (pBlueScript II) using In-Fusion PCR Cloning Kit (Takara), generating pL31 or pBS32, respectively. The URA3 gene was amplified by PCR from pJJ242 [1] and BamHI and NotI sequences were attached to the ends by two rounds of PCR as shown. The PCR product, containing 15 bp extensions homologous to the ends of BamHI-linearized pL31, was cloned into pL31 using In-Fusion PCR Cloning Kit, generating pUL31.
Next, a 3.1 kb genomic sequence, termed YF35 (sequence position 261734-264823), representing one unit of the repeat structure, was amplified by PCR from LS20 genomic DNA. BglII and BamHI sequences were attached to the ends by PCR as shown. The PCR product was cloned into pUL31 and pBS32, generating pULF31, pULR31, pF32, and pR32.
Finally, the F32 or R32 fragment was excised from pF32 or pR32 and cloned into pULF31 and pULR31, generating pULFF, pULFR, and pULRR.

Fig.S7

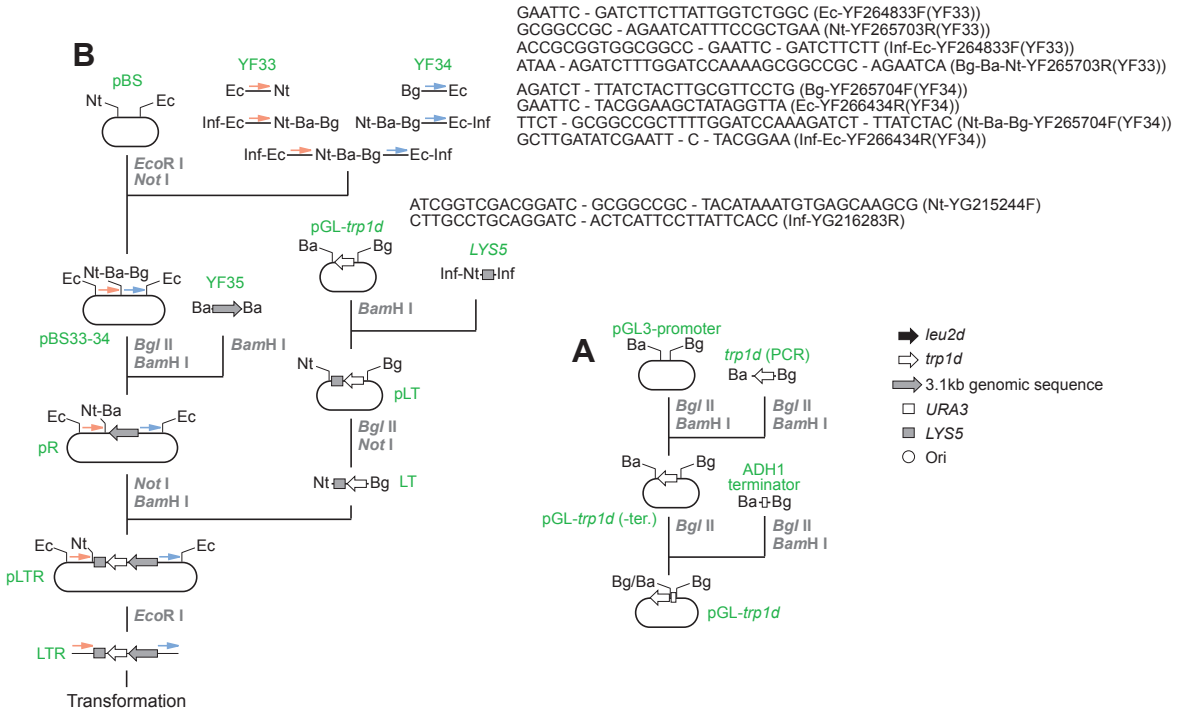

Fig. S7 Construction of pGL-trp1d and pLTR for complex repeat structures.
Restriction enzymes used for cutting plasmids are indicated in the bold gray typeface. Ba: BamHI; Bg: BglII; Ec: EcoRI; Nt: NotI. The designations of plasmids and fragments are indicated in green. The sequences and names of PCR primers are indicated to the right.
(**A**) Construction of pGL-trp1d.
The *trp1d* fragment containing the TRP1 ORF and its 5'- (30 bp) and 3'- (51 bp) flanking sequences was amplified by PCR from pJJ280 [1] using primers with BglII and BamHI sequence tags. This *trp1d* gene and a transcriptional terminator of the ADH1 gene, for insulating against read-through transcription from the upstream region, were cloned into the pGL3-promoter vector (Promega), creating pGL-trp1d. The ADH1 terminator was amplified by PCR from pAUR123 (TaKaRa) using the primer set GCTGT-BamHI-GTGTGGAAGAAC and BglII-AGAGGTTAACTAAGCGA.
(**B**) Construction of pLTR.
First, the upstream and downstream sequence (red and blue arrows) for targeting the host genomic region, termed YF33 (264833-265703) and YF34 (265704-266434), were amplified by PCR from LS20 genomic DNA. EcoRI, BglII, BamHI, and NotI sequences were attached to the ends by two rounds of PCR as shown. These PCR products have 34 bp homologous sequences at one end by the primers as shown, and were thereby assembled by PCR. The resulting PCR product, containing 15 bp extensions homologous to the ends of EcoRI-NotI-linearized pBS, was cloned into pBS using In-Fusion PCR Cloning Kit, generating pBS33-34. The YF35 fragment described in Fig. S6 was cloned into pBS33-34, generating pR.
Next, the LYS5 gene was amplified by PCR from DNA of a wild-type strain, W303, and NotI sequences were attached to the ends as shown. The PCR product, containing 15 bp extensions homologous to the ends of BamHI-linearized pGL-trp1d, was cloned into the pGL-trp1d using In-Fusion PCR Cloning Kit, generating pLT.
Finally, the LT fragment was excised from pLT and cloned into pR, generating pLTR.
From these plasmids, LTR and ULFF/ULFR/ULRR fragments were excised by EcoRI digestion and used to transform the LS20 strain.

Table S1. List of targeting primers

Primer name	Sequence	
RAD51-YE349317F	5'-CCGTTAATAGAACTTGGATCCGACA-3'	check
RAD51-YE349419F	5'-GTTCAAACTTACTTAGCAGCTTCCC-3'	targeting
RAD51-YE351860R	5'-GAAATATGAGATGAAACGGATGACCAG-3'	targeting
RAD51-YE351888R	5'-ATCATTTGTAAACACATCACCTGAGCG-3'	check
RAD52-YM211207F	5'-AAAACAGCAGTTAGTTTCCGAAGTGGT-3'	check
RAD52-YM211616F	5'-ATTAAAACCAATGACGTTCCGAGACA-3'	targeting
RAD52-YM214497R	5'-CAATTGGCCTAGAATGAAAGTAAGTGA-3'	targeting
RAD52-YM214989R	5'-CAAATTCGAAATTAGCTTGCCCTTGTG-3'	check
CHK1-YB748881F	5'-TCTTTGTTTCAGTTGTTCACGACGTTG-3'	check
CHK1-YB748994F	5'-GATTGGTTTCCCTGGACGAACTCTCA-3'	targeting
CHK1-YB751739R	5'-ATGAGTTAATGCCACCAGATTCACCTC-3'	targeting
CHK1-YB751876R	5'-ATTAATGAGCAAGTGGATAAGAGCTTCA-3'	check
MUS81-YD1245291F	5'-TTACTTTACATGCCGATGCTATCCAC-3'	check
MUS81-YD1245403F	5'-TTTCTTGGTCGAAATTCAATGTCCAG-3'	targeting
MUS81-YD1248719R	5'-GGCTTTAATGCTTTTATGTACTATGCG-3'	targeting
MUS81-YD1248739R	5'-TGTTTTTCCAGCAAATCACTGGCTT-3'	check
RAD27-YK223596F	5'-TCCTACTAAATTTCCAGTACCAGCCAT-3'	check
RAD27-YK223829F	5'-CCAAATTAATAAAGTATTGGCCATGCG-3'	targeting
RAD27-YK226108R	5'-CTAAACAAAATGCTGTCACTAACCGTA-3'	targeting
RAD27-YK226275R	5'-GCCAGGTCTTTATTAATTATAGGGTGC-3'	check
SLX1-YB674545F	5'-CGCAACATCAAATTCAGCATTTACGA-3'	check
SLX1-YB674589F	5'-CTTCAACATCTTCACCTATGTAACCTGC-3'	targeting
SLX1-YB676904R	5'-CTATGAATTCGCATCTTTACACTCT-3'	targeting
SLX1-YB676975R	5'-GGTCTATTAGTCAAACCTGTCACGGAG-3'	check
RAD18-YC230788F	5'-TTTCACCAAGAAAGTCCTCTGCACC-3'	check
RAD18-YC230913F	5'-GATGGTAACAATACTACGGATAGCAAT-3'	targeting
RAD18-YC233526R	5'-CAACAGCACCATCTTTTGTAAGTAGCC-3'	targeting
RAD18-YC233726R	5'-TGATGCTGTACAATCACCTAAGACGAG-3'	check
RAD9-YD898847F	5'-ATTTGCCGAAAAGTACCAATTATCTCTGA-3'	check
RAD9-YD899004F	5'-TTATGTCTCGTTGCTGATATGTGTCGTCC-3'	targeting
RAD9-YD904149R	5'-GTCGAAGACCAAAACACCTCCGATT-3'	targeting
RAD9-YD904205R	5'-AAAATTCGGAAAATAGGGTTGAGCA-3'	check
kanB	5'-CTGCAGCGAGGAGCCGTAAT-3'	check
kanC	5'-TGATTTTGATGACGAGCGTAAT-3'	check