Supplementary Information for

# Large-scale contractions of Friedreich's ataxia $(GAA)_n$ repeats occur during DNA replication owing to their triplex-forming potential in yeast

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**This PDF file includes:**

- Supplementary text
- Figures S1 to S10
- Tables S1 to S3

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## Supplementary Information Text

### Methods

**Fluctuation tests.** To compare the rates of contraction in the conditions of high and low transcription, the cells were first grown overnight in a liquid complete media with raffinose as a sugar source. Cells were then diluted and plated on YNB media, which contained glucose or galactose as a sugar source and was additionally supplemented with uracil and adenine. Colonies from the media with galactose were grown for 60 hours instead of 40 before plating the fluctuation test. The rest of the fluctuation test was identical to the protocol described above.

**Cloning of the cassettes.** The $P_{Gal1}$-*UR-(GAA)*$_{124}$-*A3-TRP1* cassette was constructed as previously described (1). To create cassettes with other repeats, we ordered DNA fragments from GenScript which were identical to a piece of the $P_{Gal1}$-*UR-(GAA)*$_{124}$-*A3-TRP1* cassette, except had other repeats in place of $(GAA)_{124}$ repeats. The fragments were cloned into the $P_{Gal1}$-*UR-(GAA)*$_{124}$-*A3-TRP1* cassette using *SgrAI* and *NcoI* or *SgrDI* and *BglII* restriction enzymes. To get the inverted cassettes, we amplified the direct cassettes with primers carrying *SgrDI* and *BglII* recognition sites in their 5'-tails and cloned back to the vector using the same enzymes, as previously described (1). All constructs were Sanger sequenced and integrated into yeast as linear fragments. Their integration was confirmed using PCR.

**Yeast strain construction.** All of the strains used in this study are derivatives of SMY710 (CH1585 *MATa leu2-Δ1 trp1-Δ63 ura3-52 his3-200 ade2Δ::KanMX4 HAP1-wt*) (2), except for Pol $\alpha$ mutants and their corresponding WT strain which are derivatives of CH1585 (*MATa leu2-Δ1 trp1-Δ63 ura3-52 his3-200*) (3). To construct gene knockouts, we used gene replacement with *hphMX6*, or *HIS3* as selectable markers. All knockouts were confirmed using PCR with internal primers and either external or junction primers. *Rad27* missense mutants were created via twostep transformation protocol as previously described (4). All other missense mutants were made using a simplified CRISPR-Cas9 approach (5). To overexpress the yeast RPA, a plasmid containing the *RFA1*, *RFA2* and *RFA3* genes with their flanking sequences, the *HIS3* marker and 2-micron origin of replication cloned into the pUC57 vector was ordered from *Gene Universal.* Plasmid was transformed into yeast and the cells were grown on media lacking histidine to facilitate plasmid retention. A complete list of all yeast strains used in this study can be found in the supplemental tables.

**Spot tests**. Yeast cells were plated on complete media supplemented with uracil and adenine (YPDUA) with no or 0.5 mM $H_2O_2$. After they formed colonies, the colonies were dissolved in water and 5-fold dilutions were made. 4$\mu$l of each dilution was spotted on YPDUA and $Gal^+Ura^-$ media.

**Distributions of GAA repeat sizes.** To build a distribution of repeat sizes in WT colonies without applied selection, the strains were plated to single colonies and PCR of individual colonies was performed. *Image Lab* software (*Bio-Rad*) was used to estimate the size of the repeat tracts in each sample.

To build a distribution of deleted repeats after selection, 8 to 16 colonies were randomly picked from each of the selective media. Colony PCR was performed with A2 and B2 primers. *Total Lab Count* or *Image Lab* software was used to estimate the size of the repeat tracts in each sample. If two or more colonies from the same selective plate showed an identical size of repeat deletion, that value was only accounted once.

**RT-qPCR.** Three independent colonies from each strain and condition were grown overnight. In the morning, the cells were diluted to an $OD_{600}$ of 0.1 and harvested when they reached an $OD_{600}$ of 0.5. RNA was isolated using RNeasy Kit (*Qiagen*) following the manufacturer’s instructions. Extracted RNA was treated with Turbo DNase (*ThermoFisher*) following the manufacturer’s instructions. Reverse transcription was performed using SuperScript IV Reverse Transcriptase (*ThermoFisher*) following the manufacturer’s instructions. Random hexamers and polyT primers were used to estimate URA3 transciption and RPA overexpression correspondingly. Next, qPCR was performed using SYBR Select Master Mix (*ThermoFisher*) on a QuantStudio 6 Flex Real-Time PCR system (*ThermoFisher*). To compare different samples to one another, mRNA levels were normalized to the amount of *ACT1* mRNA. Standard curves based on 10-fold serial dilution of gDNA were used for the quantification of the results. Two-sample *t*-test was used to estimate the significance.

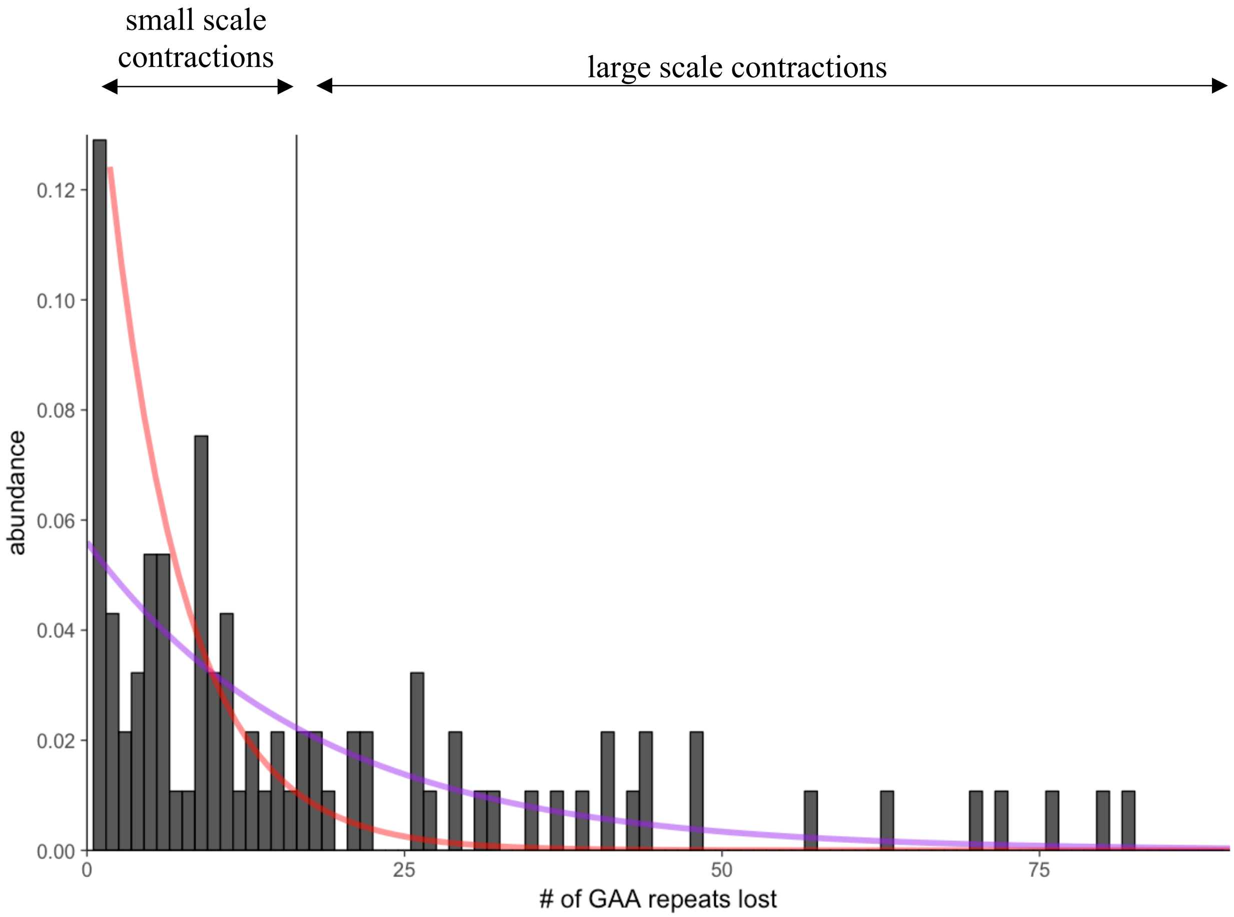

**Fig. S1.** Distribution of contracted repeats in the inverted cassette in the absence of selection. Red line represents exponential distribution fit for the distribution of all contractions (Kolmogorov-Smirnov test, P=0.13). Purple line represents exponential distribution fit for the small-scale contractions (Kolmogorov-Smirnov test, P=0.24). The vertical bar denotes the border between small and large scale contractions as classified by *k*-means clustering analysis.

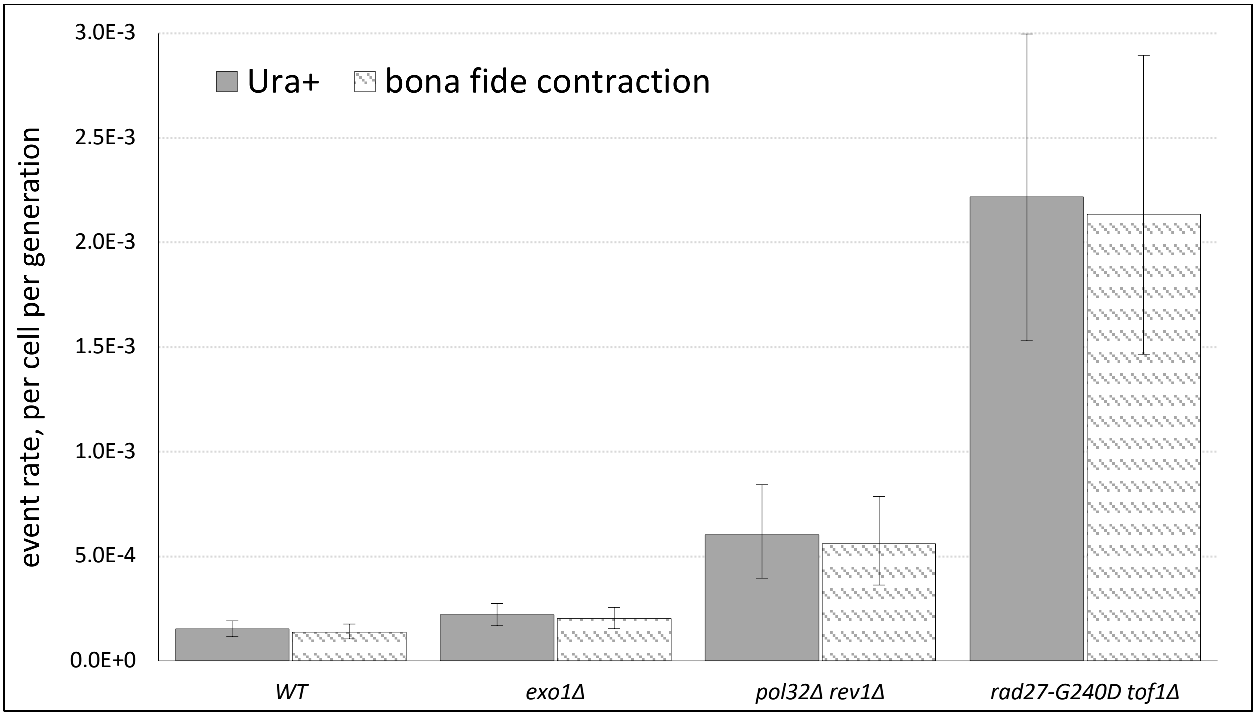

**Fig. S2.** Comparison of the Ura$^+$ and *bona fide* contraction rates for various mutants. Error bars represent 95% confidence intervals.

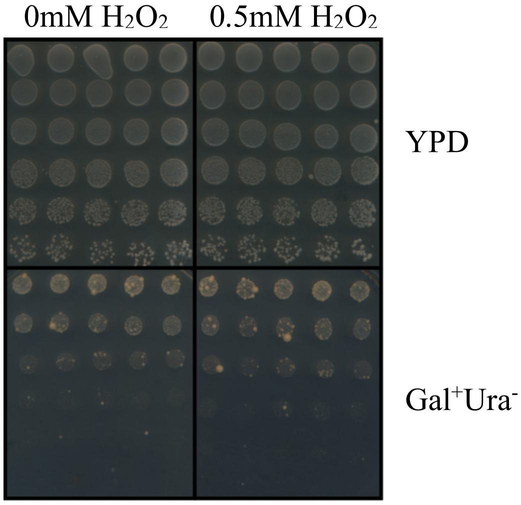

**Fig. S3.** Colonies of WT cells bearing the $(GAA)_{124}$ repeat tract in the direct cassette were grown on YPD plates supplemented with adenine, uracil, and either 0 or 0.5mM of $H_2O_2$. Figure shows 5 colonies from each plate with intact repeat tracts spotted onto complete (YPD) or selective media ($Gal^+Ura^-$).

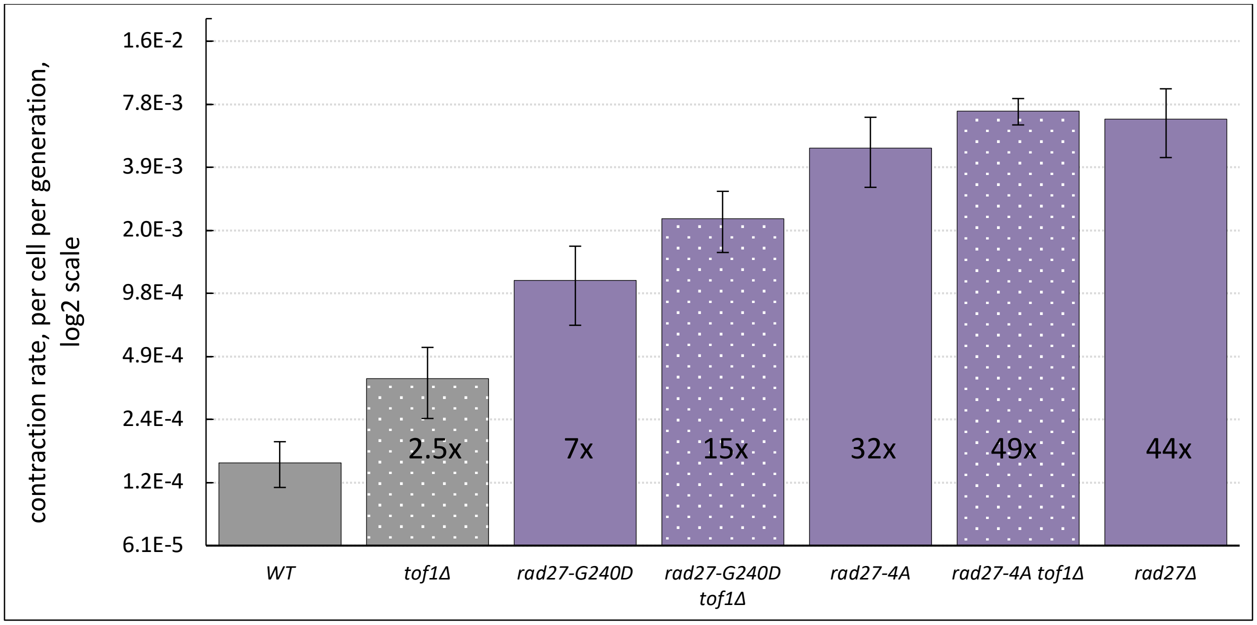

**Fig. S4.** Effect of knocking out *TOF1* on GAA repeat contraction rates in two *rad27* mutants. Error bars represent 95% confidence intervals. Numbers on bars show fold change relative to WT.

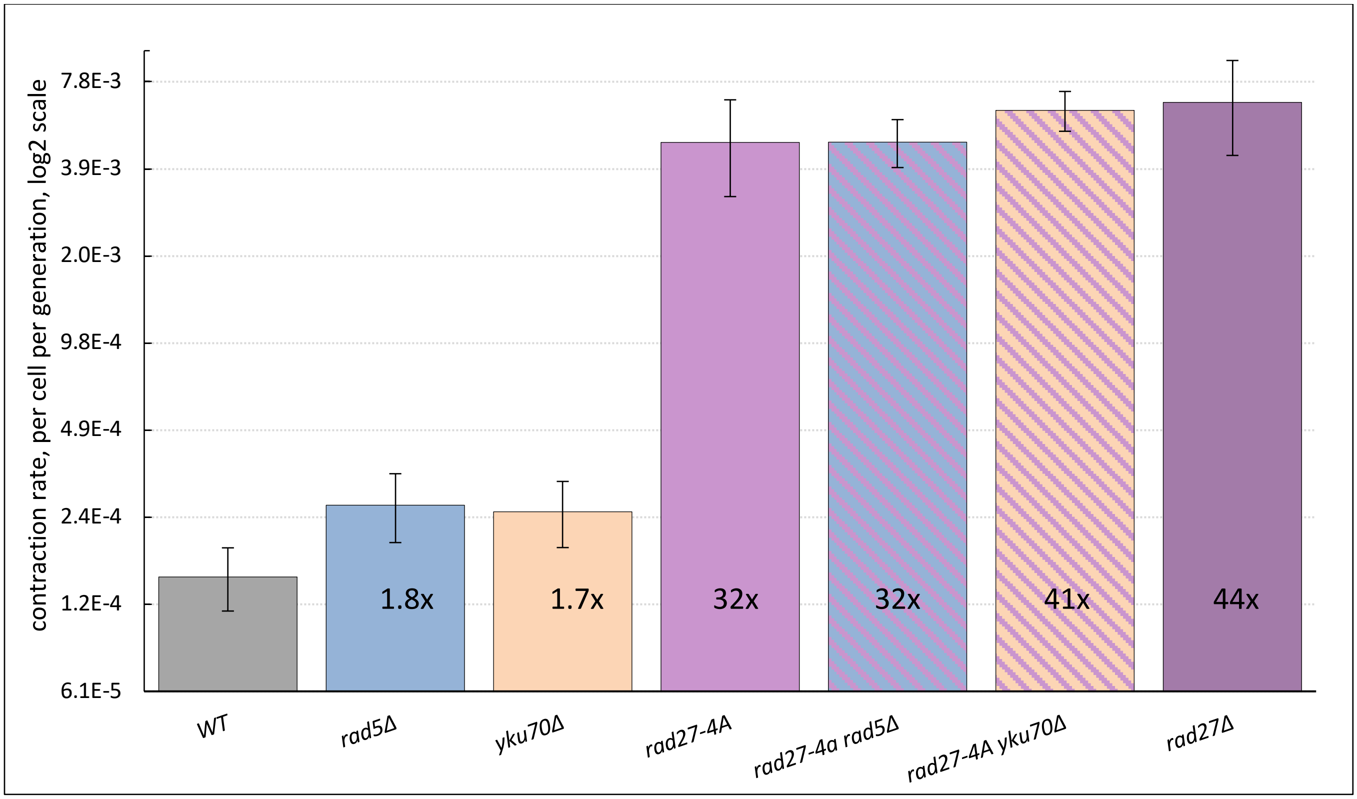

**Fig S5.** Effect of knocking out *RAD5* or *YKU70* on GAA repeat contraction rates in the *rad27-4A* mutant. Error bars represent 95% confidence intervals. Numbers on bars show fold change relative to WT.

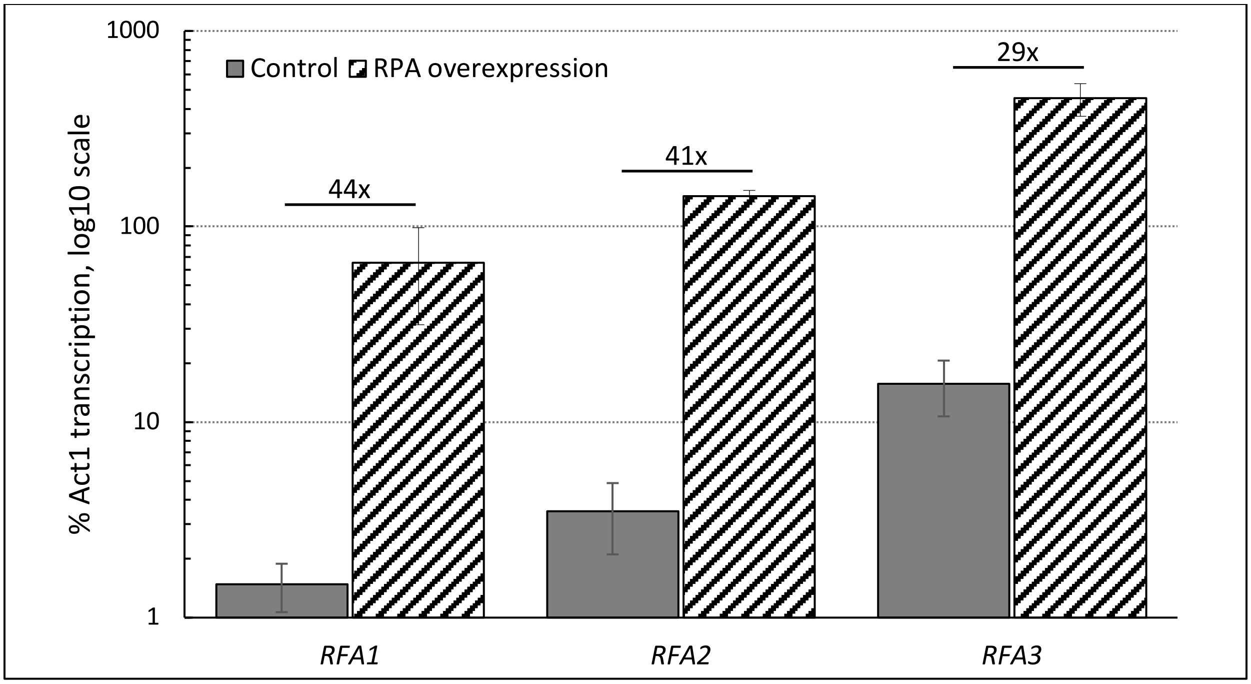

**Fig S6.** Levels of RPA overexpression. RPA overexpression was achieved by transforming yeast cells with a multicopy plasmid bearing three yeast RPA subunits. Total RNA was isolated from cells with (RPA overexpression) or without (control) the plasmid and reverse transcribed using a polyT primer. The amount of RPA cDNA was estimated using qPCR. The standard curve method was used to quantify transcription levels. Means of three biological replicates are shown and the error bars represent standart deviations. Fold changes in gene expression are shown above each bar.

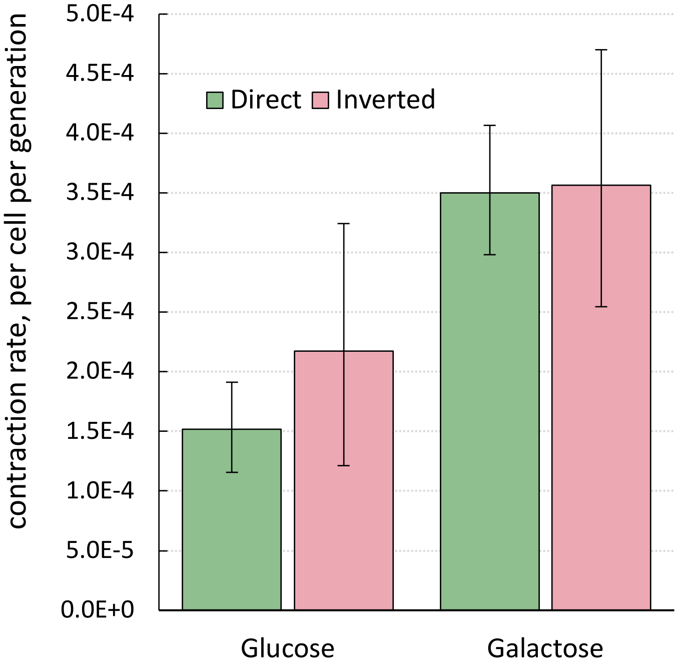

**Fig. S7.** The effect of low (glucose) or high (galactose) transcription state on GAA repeat contraction rate in strains with direct versus inverted cassettes. Bars represent 95%-confidence intervals.

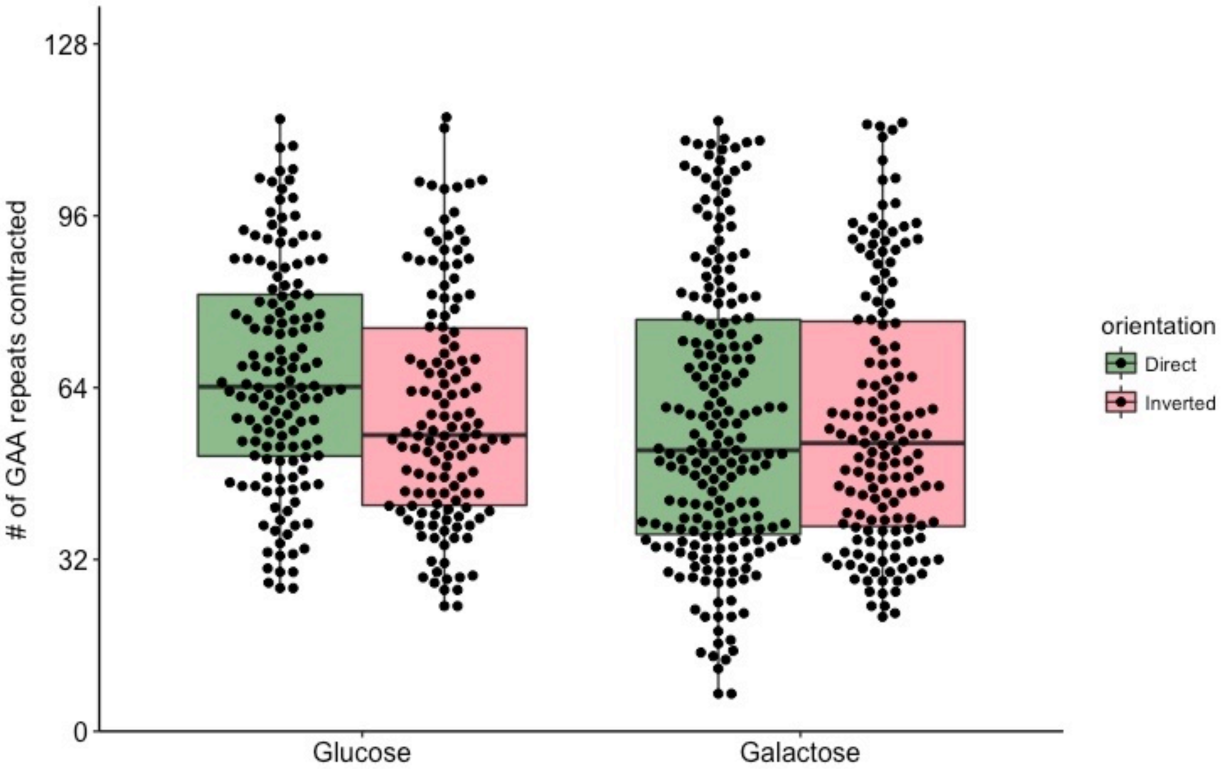

**Fig. S8.** Distribution of contraction sizes for direct versus inverted cassettes in colonies with repressed (glucose) and induced (galactose) transcription through the reporter. Each box represents median and interquartile range; whiskers show 1.5 interquartile range.

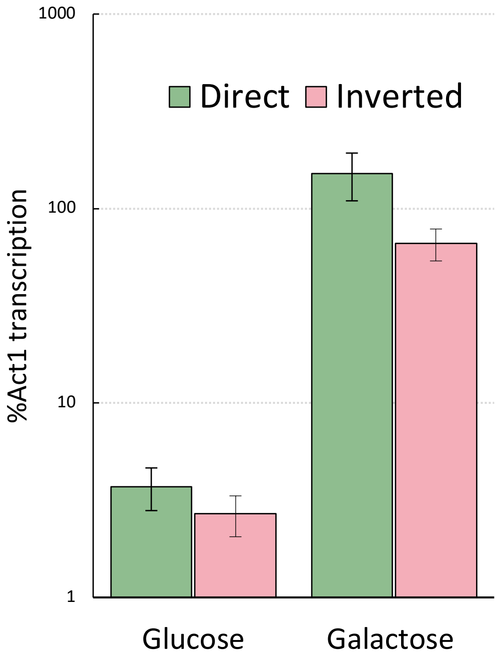

**Fig. S9**. *URA3* transcription level in cell cultures with direct versus inverted orientation of the cassette grown under transcription repression (glucose) or induction (galactose) measured by RT-qPCR. The standard curve method was used to quantify transcription levels. Mean values of three biological replicates are shown. Error bars represent standard deviations.

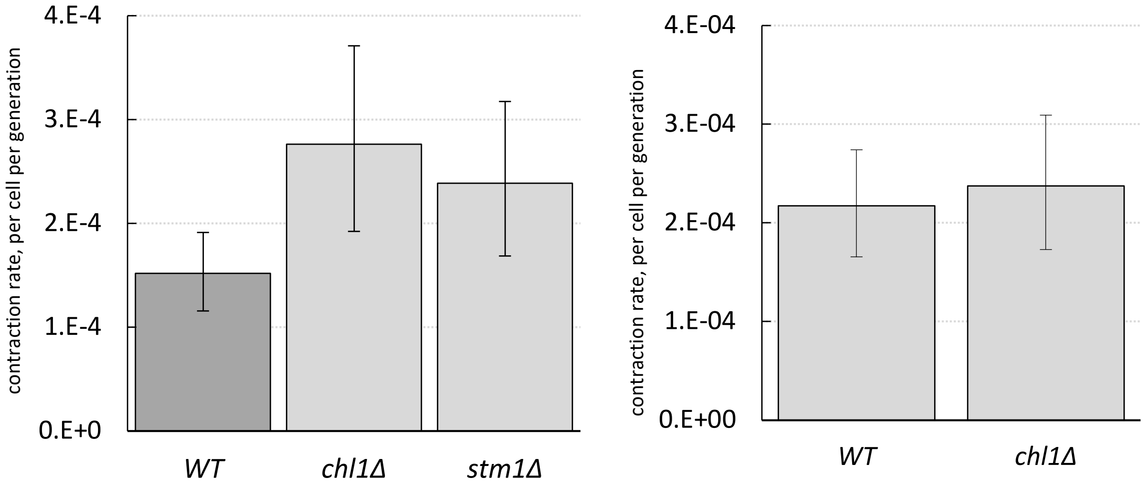

**Fig. S10** The effect of mutations in genes that were reported to interact with DNA triplex on GAA repeat contraction rates in the direct **(A)** and inverted **(B)** cassettes. Error bars represent 95% confidence intervals.

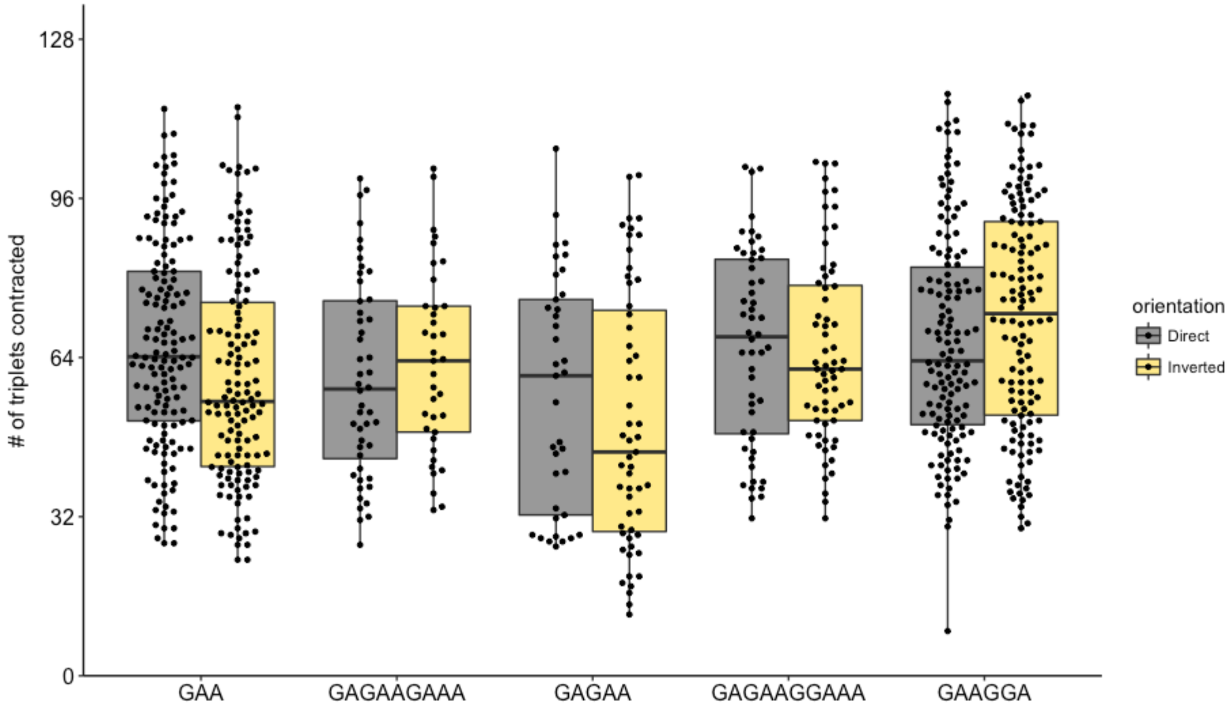

**Fig. S11**. Distribution of contraction sizes for direct and inverted cassettes depending on repeat composition. Each box represents median and interquartile range; whiskers show 1.5 interquartile range.

**Table S1.** Fraction of colonies with *bona fide* contractions among Ura$^+$ colonies for various mutants. Colonies of various strains were recoved from selective media after a fluctuation test and subjected to PCR analysis of the repeat tract lengh.

| | # of total colonies analyzed | # of *bona fide* contractions | % of *bona fide* contractions |
|---|---|---|---|
| WT | 183 | 167 | 91 |
| *rad27-G240D* | 119 | 117 | 98 |
| *rad27-4A* | 128 | 124 | 97 |
| *msh3*Δ | 159 | 145 | 91 |
| *exo1*Δ | 191 | 178 | 93 |
| *rad27-G240D tof1*Δ | 191 | 183 | 96 |
| *pol32*Δ *rev1*Δ | 126 | 116 | 92 |
| *rad27*Δ | 144 | 140 | 97 |
| *rad27-G67S* | 190 | 181 | 95 |

**Table S2.** Primers used in this project

| Primer name | Primer sequence | Notes |
|---|---|---|
| For cloning | | |
| R-SgrDI | CGGATCCGGAGATCTCGCGTGGGGATGATCCACTAG | |
| L-BglII | GACCCACGTCGACGCTAGCGGGTAATAACTGATA | |
| L-SgrDI | TAAGACCGTCGACGTCGCGTGGGGATGATCCACTAG | For cassette inversion (1) |
| R-BglII | TGCTTAAGATCTCGCTAGCGGGTAATAACTGATA | For cassette inversion (1) |
| For Sanger sequencing of the cassettes | | |
| TRP1_PPP_R | CTCTGCAAGCCGCAAACT | |
| URA3-RT-UnSpl-R | GAGCCCTTGCATGACAATTC | |
| 5'_UAS_100_1-R | ATCGAATTTGAGGTCTGCACT | |
| 5'misc-F | GATGGTACGACGGTTTGTAATAGCG | |
| Alex-URA3-RT-Spl-F | ATCCTAGTCCTGTTGCTGCCAA | |
| For amplification of the cassettes | | |
| 5'misc-F | GATGGTACGACGGTTTGTAATAGCG | |
| 3'misc-R | AAGCACTAACGATTGCGTGATGG | |
| For integration of the cassettes | | |
| A36a-R | AGGGTCGTTGCCTTCTGGT | |
| A36b-F | ACGTGTACAGTTCTCTTTACATCATC | |
| TrpS-F | TCGATTTCTGACTGGGTTGGAAG | |
| 5'_UAS_100_1-R | ATCGAATTTGAGGTCTGCACT | |
| URA3-RT-UnSpl-F | TTGACTGATCTGTAATAACCACGA | |
| For repeat tract amplification | | |
| A2 | CTCGATGTGCAGAACCTGAAGCTTGATCT | |
| B2 | GCTCGAGTGCAGACCTCAAATTCGATGA | |
| S2 | TGCTCGATGTGCAGAACCTGAAGCTTG | |
| K2 | GCTCGAGTGCAGACCTCAAATTCGATG | |
| For qPCR | | |
| A3-F | CTTCCCTTTGCAAATAGTCCTCTTCC | |
| A3-R | CAGAATTGTCATGCAAGGGCTCC | |
| Act1_F | CTCCACCACTGCTGAAAGAG | |
| Act1_R | GTGATGACTTGACCATCTGG | |
| RFA1_qPCR_F | TCGTGTGACGGATTTTGGTGG | |
| RFA1_seq_R2 | GATGAAGTTTGCGTTGCGGC | |
| RFA2_qPCR_F | TCACGTTTGTTTTGTAGGTGTGG | |
| RFA2_seq_R | CATCGTTACCGGCAGCCAAG | |
| RFA3_seq_F | CGAAACACCAAGAGTTGACCCC | |
| RFA3_qPCR_R | CGTAATCATTTCAACCTCGCTGCC | |
| For amplification of the pRCC-N plasmid with target gRNA sequence | | |
| DNA2_H547A_pRCCN_F | TATTGCCTAAAGTCATGACAGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGG | *dna2_H547A* |
| DNA2_H547A_pRCCN_R | TGTCATGACTTTAGGCAATACGATCATTTATCTTTCACTGCGGAG | *dna2_H547A* |
| Rev1_pRCC_F | TTGATGAAGCTGTTTGTGTGGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGG | *rev1-D467A-E468A (CD)* |
| Rev1_pRCC_R | CACACAAACAGCTTCATCAACGATCATTTATCTTTCACTGCGGAG | *rev1-D467A-E468A (CD)* |
| RFA1_S351P_pRCCN_F | TCACAATTGTTGACGACTCTGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGG | *rfa1-S351P* |
| RFA1_S351P_pRCCN_R | AGAGTCGTCAACAATTGTGACGATCATTTATCTTTCACTGCGGAG | *rfa1-S351P* |

| | | |
|---|---|---|
| RFA1_K45E_pRCC_F | ATAACACCAGGAAATCTGATGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGG | *rfa1-K45E* |
| RFA1_K45E_pRCC_R | ATCAGATTTCCTGGTGTTATCGATCATTTATCTTTCACTGCGGAG | *rfa1-K45E* |
| Pol3_D520V_pRCC_F | CATAAGCCTTAAAGGCAGGTGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGG | *pol3-D520V* |
| Pol3_D520V_pRCC_R | ACCTGCCTTTAAGGCTTATGCGATCATTTATCTTTCACTGCGGAG | *pol3-D520V* |
| For amplification of a template for repair in CRISPR-Cas9 gene editing | | |
| DNA2_H547A_mut_F | AAGACGTTCAATTCTGCAAATGCAATTTCAAGATCCCCGCGGAGAACCAAGTCTTGTCATGACTTTAGGCAATATCGTAGCCGAGTTATTGCAAGACTC | *dna2_H547A* |
| DNA2_R | CTTCAATATCAATCACATTGGATATAGAAATTGGCTGTGTTCTTCTTGTTCCGGAA | *dna2_H547A* |
| REV1_mut_F | TACTCTCAAAAGATTGAACATATTCAATTTGATTTTACCTATATCTATTgcagctGCTGTTTGTGTGcgtATAATCCCTGATAA | *rev1-D467A-E468A (CD)* |
| REV1_R | TGTGCAACCATTCGTTCCTTGA | *rev1-D467A-E468A (CD)* |
| RFA1_S351P_mut_F | ATTTTGAGCTAACTTCAAGGGCTGGGAAGAAATTCGATCGTCGTGACATCACAATTGTTGACGACCCTGGCTTTTCTATCTCTGTTGGCCTATGGAATC | *rfa1-S351P* |
| RFA1_R2 | AGGCATATGCCTCAGGAATTTCTGGATTCGGAATCAGGG | *rfa1-S351P* |
| RFA1_K45E_F | GTACGATAATCCCACCGGTGGCGTTTATCAAGTTTATAACACCAGGAAATCTGATGGCGCTAACAGCAACAGAGAGAATTTGATCATGATTTCCGATGG | *rfa1-K45E* |
| RFA1_R | CTGACAATAGCAGGTTCTGCAATTATCACGCGAATGATATCACCCC | *rfa1-K45E* |
| POL3_D520V_template | AATGGCGATAGTGAAACAAGAAGAAGGTTGGCCGTTTACTGTTTGAAAGTCGCATACCTGCCTTTAAGGCTTATGGAAAAACTAATGGCGTTAGTTAAC | *pol3-D520V* (ssDNA template) |
| For Sanger sequencing of the missense mutations | | |
| DNA2_seq_R | CCTTGAACCTCGTATGAAACGCTTC | *dna2_H547A* |
| DNA2_seq_F | GCTAACGACAACCTGTTGGTGC | *dna2_H547A* |
| REV1_F | AATGTGTAGGGTCGGCATTG | *rev1-D467A-E468A (CD)* |
| REV1_R | TGTGCAACCATTCGTTCCTTGA | *rev1-D467A-E468A (CD)* |
| RFA1_seq_F2 | CTCCAACCAGCTAAGCCCCA | *rfa1-S351P* |
| RFA1_seq_R2 | GATGAAGTTTGCGTTGCGGC | *rfa1-S351P* |
| RFA1_seq_F | ACACACCACAAATACCCCATTTCC | *rfa1-K45E* |
| RFA1_seq_R | CGACTGGACCAACTCAAAGTCATC | *rfa1-K45E* |
| POL3_seq_F | GTGGTTTTCTAACGGCACGACT | *pol3-D520V* |
| POL3_seq_R | CATATTGGTCATCAGAGGCCTGAG | *pol3-D520V* |
| RAD27_seq_F | TAACATCGCGCAAATGAAGG | |
| For knockout fragments amplification | | |
| APN1_pRS_F | ATGCCTTCGACACCTAGCTTTGTTAGATCTGCTGTCTCGAAATACAAATTTGGTGTCGGGGCTGGCTTAA | *apn1::HIS3* |
| APN1_pRS_R | TTTGTTTAACCTCAAACTTGTCTAACTGTTCCTTACGCGATTTAGCACCTGTTTACAATTTCCTGATGCGGTA | *apn1::HIS3* |
| CHL1-pRS-F | CCAAAAAATGGACAAAAAGGAATATTCGGAGACTTTCTATCATCCTTATAAGCCCGGTGTCGGGGCTGGCTTAA | *chl1::HIS3* |

| | | |
|---|---|---|
| CHL1-pRS-R | ATCACAGTATACACGTAAACGTATTCCTTTTAGCGTGAATTCAGGCTGCGCATTGGTTTACAATTTCCTGATGCGGTA | *chl1::HIS3* |
| EXO1_pRS_F | GAAAGGAATGGGTATCCAAGGTCTTCTTCCTCAGTTAAAGCCCATACAGAGGTGTCGGGGCTGGCTTAA | *exo1::HIS3* |
| EXO1_pRS_R | GATTTTCATTTGAAAAATATACCTCCGATATGAAACGTGCAGTACTTAACGTTTACAATTTCCTGATGCGGTA | *exo1::HIS3* |
| MLH1-pAG-F | ATGTCTCTCAGAATAAAAGCACTTGATGCATCAGTGGTTAACAAAATTGCCAGCTGAAGCTTCGTACGC | *mlh1:: HphMX4* |
| MLH1-pAG-R | TTAACACCTCTCAAAAACTTTGTATAGATCTGGAAGGTTGGCTATTTCCACATAGGCCACTAGTGGATCTG | *mlh1:: HphMX4* |
| MLH2_pRS_F | CACATCCCATCATCTCGGTTTGAGGAACAGACGCCTTTTCATAGTTTTGGGGTGTCGGGGCTGGCTTAA | *mlh2::HIS3* |
| MLH2_pRS_R | TCTATTATGAAGTAATCTATTGTGCTGAGTGGTGATAGTGCACCCGATCAGTTTACAATTTCCTGATGCGGTA | *mlh2::HIS3* |
| MLH3_pRS_F | CATAAACCAGCGAGGCTTTCAAGGAAGAATGAACGTGAACTCGTCAACTCGGTGTCGGGGCTGGCTTAA | *mlh3::HIS3* |
| MLH3_pRS_R | GCGCAATTTAAAATGCAGGCGACAAACCTTGTTCCAGGATTAAGGTTCTCGTTTACAATTTCCTGATGCGGTA | *mlh3::HIS3* |
| JK230_MRE11_SP | TTAAGAGAATGCAGACAATTGACGCAAGTTGTACCTGCTCACATCCGATAAAACTCGACTCAGCTGAAGCTTCGTACGC | *mre11::HphMX4* |
| JK321_MRE11_ASP | TCGCGAAGGCAAGCCCTTGGTTATAAATAGGATATAATATAATATAGGGATCAAGTACAACATAGGCCACTAGTGGATCTG | *mre11::HphMX4* |
| KMH2_F | GACACTCTACTCCAATATCAACTGTAAAAAACTCTTTATCTGCTGACCTAACATCAAAATCCTCAGATCTGTTTAGCTTGCCTCGTC | *msh2::HphMX4* |
| KMS2_R | CCAAAAGACTGATCTGAAATACCAGGCTCAACTTTGTATAACAACGTGATGTCCTCATCGATGAATTCGAGCTCGTTTTCG | *msh2::HphMX4* |
| JK215_MSH3_SP | GTACTTTTGAGAGCCAAAAGCAGTGCAAATAGATTTATTTTGTTGAATCTATTAACAATACAGCTGAAGCTTCGTACGC | *msh3::HphMX4* |
| JK216_MSH3_ASP | GCATAAGAAATTGCTATACCATCGTGCGTGCCAGTACCTCTTCCCACTTCGTCTAATAATCATAGGCCACTAGTGGATCTG | *msh3::HphMX4* |
| JK217_MSH6_SP | GATAAGATTTTTTAATTGGAGCAACTAGTTAATTTTGACAAAGCCAATTTGAACTCCAAACAGCTGAAGCTTCGTACGC | *msh6::HphMX4* |
| JK218_MSH6_ASP | TAAAGCATGGATGTCTTAATGATTTAAATTTCAGAAAACCATTTAATTGAGTATTCGTTTCATAGGTTACTAGTGGATCTG | *msh6::HphMX4* |
| JK246_MUS81_SP | ACATTGGCGTAAACAAAGTTTCAAAGGATTGATACGAACACACATTCCTAGCATGAAAGCCAGCTGAAGCTTCGTACGC | *mus81::HphMX4* |
| JK247_MUS81_ASP | AAAGAATATCATCACTTTTTTCTTTATAAAACCTTGCAGGGATGACTATATTTCAAATTGCATAGGCCACTAGTGGATCTG | *mus81::HphMX4* |
| OGG1_F | ACTACTATTTCCAGCGGAAGAAGGCATTTGAAGCGTCCTGATTCATAATTGCGATCGGATCCCCGGGTTAATTAA | *ogg1::HphMX4* |

| | | |
|---|---|---|
| OGG1_R | TTTGCTTCTTTGATGTGAAGATCAGACAATTCAACTTTCAGTTTCATTTGTTTCGCATAGGCCACTAGTGGATCTG | *ogg1::HphMX4* |
| PMS1_F | GATGTTCATCGAATTACATCTGGACAAGTTATCACCGACTTAACAACTGCCGGATCCCCGGGTTAATTAA | *pms1::HphMX4* |
| PMS1_R | GGACAATTCCAAGGCTTATCAAGTTCACTGAGATTATGAACGACTCTGGTCATAGGCCACTAGTGGATCTG | *pms1::HphMX4* |
| JK219_POL32_SP | ATAATATTTCACATTAACTAACAACCAGAAATAGGCTTTAGTTAACTCAATCGGTAATTACAGCTGAAGCTTCGTACGC | *pol32::HphMX4* |
| JK220_POL32_ASP | CATTTGTATTATACATTACATCACAATTAGTAATGGAAAGTGTTTGGAAAAAAAAGAAGACATAGGCCACTAGTGGATCTG | *pol32::HphMX4* |
| RAD14_F | AGAGTTTGGATCTTCGTAGTGAAGGTATCGAACGTAACGCTATGACTCCCCGGATCCCCGGGTTAATTAA | *rad14::HphMX4* |
| RAD14_R | TATACATAACCAACATTTAAATGTCAATTTCTTCAGTTTCTAGCCCGCAGCATAGGCCACTAGTGGATCTG | *rad14::HphMX4* |
| RAD18_pRS_F | ATGGACCACCAAATAACCACTGCAAGCGACTTCACGACTACTTCAATACCGATCTTGGCCTCCTCTAGTACACTC | *rad18::HIS3* |
| RAD18_pRS_R | TTAATTGTTACCGGGTGGGTCTTTACTATATTCATTCAAGTCCATTAATTCTGTTTACAATTTCCTGATGCGGTA | *rad18::HIS3* |
| RAD2_Hyg_Int_F | CTAGTATAACCCATTCGAACCTCCGTGGAGGCATTAAAAGGGAGAGTGAAATTAGAACGCGGCTACAATTA | *rad2::HphMX4* |
| RAD2_Hyg_Int_R | AAGGACCGTATATATCTACTATTCCTGGATCGGTTGACTTTGTTAACATGCAGAAACACCCTGATTCTGTGGATAACC | *rad2::HphMX4* |
| JK272_RAD27_SP | TATACATCGATGAAAAGCGTTGACAGCATACATTGGAAGAAATAGGAAACGGACACCGGAAGAAAAAATCAGCTGAAGCTTCGTACGC | *rad27::HphMX4* |
| JK273_RAD27_ASP | GTATACAAATACTATGTTACATATATGCCAAGGTGAAGGACCAAAAGAAGAAACTGGAAAAAGAACCCCCCATAGGCCACTAGTGGATCTG | *rad27::HphMX4* |
| RAD30-pRS-F | TTTTGAACGGCTTTGATAAAACAAGACAAAGCATGTCAAAATTTACTTGGGGTGTCGGGGCTGGCTTAA | *rad30::HIS3* |
| RAD30-pRS-R | ATTATCAGGACGTTTTAGTTGCTGAAGCCATATAATTGTCTATTTGGAATGTTTACAATTTCCTGATGCGGTA | *rad30::HIS3* |
| RAD5del_Hyg_F | CCTTACTGCTAAGCGCATTGCTCACTTGAAAGTAAATTATCTACAAAGTTACACATACGATTTAGGTGAC | *rad5::HphMX4* |
| RAD5del_Hyg_R | TCTATGCTATCTTGTATGATAAATCTCATAACTTTGACGCTGTTTGTCTGTCTGTGGATAACCGTATTAC | *rad5::HphMX4* |
| RAD5_pRS_F | CCTTACTGCTAAGCGCATTGCTCACTTGAAAGTAAATTATCTACAAAGTTACATTCGGTGATGACGGTGAAA | *rad5::HIS3* |
| RAD5_pRS_R | TCTATGCTATCTTGTATGATAAATCTCATAACTTTGACGCTGTTTGTCTGGTTTACAATTTCCTGATGCGGTA | *rad5::HIS3* |
| RAD51_Hyg_Int_F | AAATGTTGGAAATGCACCACTACCGTTCTTCAACCAATCTAGTTTAGCTATTTAGAACGCGGCTACAATTA | *rad51::HphMX4* |
| RAD51_Hyg_Int_F | AAAGAGGAGAATTGAAAGTAAACCTGTGTAAATAAATAGAGACAAGAGACCAAATACCTACCCTGATTCTGTGGATAACC | *rad51::HphMX4* |
| RAD52_pAG_R | GGTTTCACGCGGTACTTGATTCCCAGCCCCTTCTAGCATATGAGGCCCCAGTTCTTTATCATCGATGAATTCGAGCTCGTT | *rad52::HphMX4* |

| | | |
|---|---|---|
| KR52t-F | CGAATGGCGTTTTTAAGCTATTTTGCCACTGAGAATCAACAAATGCAAACAAGGAGGTTGCCAGATCTGTTTAGCTTGCCT | *rad52::HphMX4* |
| RAD59_pAG_F | GGTTACGTAGAGGAGAAGAGCATATTTCAGGATAAACAGACAAAATAATGCAGCTGAAGCTTCGTACGC | *rad59::HphMX4* |
| RAD59_pAG_R | GTGAAAATTATGACTTTTTATCAAGCAAAATAAATTTGCTACTTGTGCCCCATAGGCCACTAGTGGATCTG | *rad59::HphMX4* |
| RAD6S_HIS | CATGTCCACACCAGCTAGAAGAAGGTTGATGAGAGATTTTAAACGTATGAAGGAAGGTGTCGGGGCTGGCTTAA | *rad6::HIS3* |
| RAD6A_HIS | TCAGTCTGCTTCGTCGTCGTCGTCGTCGTCATCATCATCATCATCATCGTCCATCTCCTTACGCATCTGTGCGGTA | *rad6::HIS3* |
| REV1_pRS_F | ACAGATTTTCTCAAAATAAATCGATACTGCATTTCTAGGCATATCCAGCGGGTGTCGGGGCTGGCTTAA | *rev1::HIS3* |
| REV1_pRS_F | GATATTACAGGTAATGTTCGCAAACTGCGTGTTTACTGTATGCTGAAATGGTTTACAATTTCCTGATGCGGTA | *rev1::HIS3* |
| REV3_pAG_F | ATGTCGAGGGAGTCGAACGACACAATACAGAGCGATACGGTTAGATCATCCGGATCCCCGGGTTAATTAA | *rev3::HphMX4* |
| REV3_pAG_R | GCGAGACATATCTGTGTCTAGATTACCAATCATTTAGAGATATTAATGCTGCATAGGCCACTAGTGGATCTG | *rev3::HphMX4* |
| REV3_pRS_F | ATGTCGAGGGAGTCGAACGACACAATACAGAGCGATACGGTTAGATCATCGGTGTCGGGGCTGGCTTAA | *rev3::HIS3* |
| REV3_pRS_R | GCGAGACATATCTGTGTCTAGATTACCAATCATTTAGAGATATTAATGCTGTTTACAATTTCCTGATGCGGTA | *rev3::HIS3* |
| Ksg1t-F | GGTGATCATTGGTGATACASTTTCGGATTTGTGGCTTTACCGTTTAGTTTGTTTTTATCAGCCAGATCTGTTTAGCTTGCCT | *sgs1:: HphMX4* |
| Ksg1h-R | CGCACCAGTGATGGCTAATGCCTTAGTGACGGTAGTCGCAGTAGTACTTGTCAGGTTTGAATTCGAGCTCGTTTTCGACA | *sgs1:: HphMX4* |
| JK180_SRS2_SP | TCTGCACTTTGAGTATCATTCCAATTTGATCTTTCTTCTACCGGTACTTAGGGATAGCAACAGCTGAAGCTTCGTACGC | *srs2:: HphMX4* |
| JK181_SRS2_ASP | CTTTGGCACCGTGAATTGTAGATATCGTGACAAACCCATTCTTCTCACGTTTTATTTTTGCATAGGCCACTAGTGGATCTG | *srs2:: HphMX4* |
| STM1-pAG-F | CAAAGATGGCAAGTTAGAAACGTCAATGTTACGGTTCTTTTGAACGGTGTCATAGGCCACTAGTGGATCTG | *stm1::HphMX4* |
| STM1-pAG-R | ATAGTGTCTTATCTAACAATCACTTCTGTGCAAAGTTCTAATTGTCTGGTCAGCTGAAGCTTCGTACGC | *stm1::HphMX4* |
| TOF1_SP | GTAAGTCGCCTCACATATGATAATACCATCTAGCTTGTGGGGTTTAGTGTATCTTCAGCTGAAGCTTCGTACGC | *tof1::HphMX4* |
| TOF1_ASP | TCTGTAGCTCTTATGCTTTCAATACTTGGTATGGATCCACCAAACAAGCTCGTATCATAGGCCACTAGTGGATCTG | *tof1::HphMX4* |
| TOF1-pRS_F | GTAAGTCGCCTCACATATGATAATACCATCTAGCTTGTGGGTTCGGTGATGACGGTGAAA | *tof1::HIS3* |
| TOF1-pRS_F | TCTGTAGCTCTTATGCTTTCAATACTTGGTATGGATCCACCGTTTACAATTTCCTGATGCGGTA | *tof1::His3* |
| YKU70_SP | GCGCTCAGTCACTAATGCATTTGGCAATAGTGGAGAACTTAACGATCAAGTGGATCAGCTGAAGCTTCGTACGC | *yku70::HphMX4* |
| YKU70_ASP | GCCTTTGGATGATTGGATCTTCTGACTTCTCCAGATTCTAAAATTTTATTTCGCATAGGCCACTAGTGGATCTG | *yku70::HphMX4* |

| | | |
|---|---|---|
| YKU70-pRS_F | GCGCTCAGTCACTAATGCATTTGGCAATAGTGGAGAACTTAACGATCAAGTGGATTCGGTGATGACGGTGAAA | *yku70::HIS3* |
| YKU70-pRS_R | GCCTTTGGATGATTGGATCTTCTGACTTCTCAGATTCTAAAATTTTATTTCGGTTTACAATTTCCTGATGCGGTA | *yku70::HIS3* |
| For knockout verification | | |
| APN1_chk_F | CTGGGAACTTGAACGTGGA | *APN1* |
| APN1_chk_R | GGGCAACAGCATCTTGGA | *APN1* |
| APN1_int_F | TGAAAGGAGACCATCAGTTGC | *APN1* |
| APN1_int_R | ATGTATCTATGCAAACGCCGA | *APN1* |
| CHL1-upstr-F | GGCACTACTGCAACTTCAGT | *CHL1* |
| CHL1-F | CCCTCGTTCCCATCATCCTT | *CHL1* |
| CHL1-R | CGTATCAGGACAATGACGCC | *CHL1* |
| EXO1_KOcheckFwd | GTATTACGTCCAAACTAAGTTCGCG | *EXO1* |
| EXO1_KOcheckRev | GACCGCTAGCGGCTTGATTAG | *EXO1* |
| EXO1_F | CAGCGGGAGGGAAAACTGAT | *EXO1* |
| EXO1_R | CTCTGTTGGCTAGAGGTTGGTG | *EXO1* |
| MLH1-upstr-F | GTAATCGCGCTAGCATGCTA | *MLH1* |
| MLH1-F | GCGTTGATGGAAAGGTGTGT | *MLH1* |
| MLH1-R2 | CAATGGCAGATAATTCGGCG | *MLH1* |
| MLH2_upstr_F | CATATCCCTCATATACATGGCCC | *MLH2* |
| MLH2_dnstr_R | GTGCGGTTACCATGAGTTAC | *MLH2* |
| MLH2_F | TGAACTTGGAGACGGGGAAGAC | *MLH2* |
| MLH2_R | CGGGTGTAGGTATCACCAGTGC | *MLH2* |
| MLH3_upstr_F | TTTGCGTTTATTTGCGAGCG | *MLH3* |
| MLH3_dnstr_R | GGTTTTGACAACGTGATGAGG | *MLH3* |
| MLH3_F | GTTTGGGGCAATAATACCACCGG | *MLH3* |
| MLH3_R | CGAACATCAAGGATGAAGACGGG | *MLH3* |
| JK232_MRE11_fwd | CCAATCATTTCGACCGTCACTC | *MRE11* |
| JK254_MRE11_int_fwd | TGGATATACTTCATGCGACTGG | *MRE11* |
| JK255_MRE11_int_rev | ATGACCCCATATCACCATATCC | *MRE11* |
| MSH2_upstr_F | GCACTCCATCAAGTGAACCT | *MSH2* |
| MSH2_dnstr_R | TCGTTCGGACCTAACATCTC | *MSH2* |
| MSH2_F | CATCCCATGGATTCGGAAAG | *MSH2* |
| MSH2_R | CAGCTTCTTCACAAGGTACG | *MSH2* |
| JK222-MSH3-rev | AAGGGGCAGTCACTTAACTCAG | *MSH3* |
| MSH3_F | GATTTACCACTCCCAGAACCCA | *MSH3* |
| JK221_MSH3_fwd | GTGTTCAAATCACGGTATGTGG | *MSH3* |
| MSH6-F | GGCTCGATAGTGTTGACTCTTT | *MSH6* |
| JK223_MSH6-fwd | TGACATAATGAATGGCTTCTGG | *MSH6* |
| JK224_MSH6_rev | CCCGTTAACAATCCTAATCTGG | *MSH6* |
| JK248_MUS81_fwd | AGAGGTGGTGGTCAAATCATCC | *MUS81* |
| JK249_MUS81_rev | ACTGCCTCCAATTTTGATTGCC | *MUS81* |
| JK260_MUS81_int_fwd | CACAGCAAATCTGACTGACCTC | *MUS81* |
| JK261_MUS81_int_rev | TTCGAAATCACCACTACACCAC | *MUS81* |
| OGG1_chk_F | CGCCTTTCTTAATGTAACGCC | *OGG1* |
| OGG1_chk_R | GCTCCTTTAAAGAATATGTATCGCC | *OGG1* |
| OGG1_in_chk_F | GCTCTAATTTCGGGAACTTAATCAC | *OGG1* |
| OGG1_in_chk_R | AGATACTTTGAAGGTATGTGGTGTC | *OGG1* |
| PMS1_chk_F | GAACAGAGGTATATCCCTGTGAAA | *PMS1* |
| PMS1_chk_R | CCATCAAGCATCTTCAATGCAC | *PMS1* |
| PMS1_chk_in_F | CTCACAATCAGAACAACAAGCTC | *PMS1* |

| | | |
|---|---|---|
| PMS1_chk_in_R | TTAGTCGGTGTACTTGAGTTGC | *PMS1* |
| JK225_POL32_fwd | TTTCCACTACGGTGTAACTTTCC | *POL32* |
| POL32_in_F | GACCACGCCAGAAGAAACAA | *POL32* |
| POL32_in_R | GCTGTCGTTTCCAACAAGTC | *POL32* |
| RAD14_chk_F | CGTTTGCTAAGTTGTAGGGAGA | *RAD14* |
| RAD14_chk_R | GTACGAGTGACAAATGGGATATCA | *RAD14* |
| RAD14_chk_in_F | CCGATGACCAAGAATTTGAATCTG | *RAD14* |
| RAD14_chk_in_R | CTGGATGCTCCTTAGAACACTG | *RAD14* |
| RAD18_upstr_F | GAGCAATGCCACATTAGAAG | *RAD18* |
| RAD18_dnstr_R | GTGCACAAGCTAACAAACAG | *RAD18* |
| RAD18_F | CCACTGAGTTCCAAACCATC | *RAD18* |
| RAD18_R | GACTTCTGGAGTTCGTACCT | *RAD18* |
| RAD2_chk_F | TTGATGTTTCCAGAGGATGTGA | *RAD2* |
| RAD2_F | ACCGTCTCAGCAGGAGGATA | *RAD2* |
| RAD2_R | GAGATTTAGTGGGAACGTCCTC | *RAD2* |
| JK274_RAD27_fwd | GACTAGTACCCGGCTGAATCAC | *RAD27* |
| JK275_RAD27_rev | CTGTGTATACTGCGGAGAAACC | *RAD27* |
| JK276_RAD27_int_fwd | CAAAGAAGGGAAAGGTGTATGC | *RAD27* |
| JK277_RAD27_int_rev | CCCCAGATTCAATAAACTCCAC | *RAD27* |
| RAD30_upstr_F | TTGTAGTCTTCTAGCGCAGG | *RAD30* |
| RAD30_dnstr_R | GCGCCCGTGAATCATTTAGA | *RAD30* |
| RAD30_F | CGTGTGGTTTGTCTAGCACC | *RAD30* |
| RAD30_R | CAGACCGTACCGGCCTCTAC | *RAD30* |
| RAD5_del_chk_F | TTACGCGTCATAAACCCTT | *RAD5* |
| RAD5_del_chk_R | ACAGCATCTGGATTTCTTCA | *RAD5* |
| RAD5_in_F | CTGAAGTCCATAACAATCTCCGA | *RAD5* |
| RAD5_in_R | GCCATTTGAACTGCTTCCATAA | *RAD5* |
| R51TF | AGTACGCGTGGTGGGACCATA | *RAD51* |
| R51TR | GGACCCTGCAGGAGGAAGTA | *RAD51* |
| RAD51_chk_in_F | AGATCGGAGCTGATTTGTTTGAC | *RAD51* |
| RAD51_chk_in_R | CTTCACCGCCACCAATATCC | *RAD51* |
| Ex52_R | ACGTCGCTAAAGATGGTATGGTA | *RAD52* |
| RAD52_chk_in_F | GCCAAGAAATCTGCCGTTAC | *RAD52* |
| RAD52_chk_in_R | TGAGCTTTCGCTGATTTCATCC | *RAD52* |
| RAD59_upstr_F | GCAAGGGCAGATATGATAGG | *RAD59* |
| RAD59_dnstr_R | CCTTCGTTACCTTGGAATGG | *RAD59* |
| RAD59_F | TTCGACTACATACGGCACAG | *RAD59* |
| RAD59_R | GCTTGCTATTAGTCGCTGAC | *RAD59* |
| RAD6_TS | GCCGGAGTAGAAAGCTGGAA | *RAD6* |
| RAD6_TA | AAAGATACGGGTATCGGCAGTT | *RAD6* |
| RAD6_F | GGGTGTATCTGCTTCACCAT | *RAD6* |
| RAD6_R | ATCGTCCATATCATCCTCCC | *RAD6* |
| REV1_upstr_F | TACGGCAACCTTTAAGCACC | *REV1* |
| REV1_upstr_R | GAGTCGGCCATTCCAATACC | *REV1* |
| REV1_F | AATGTGTAGGGTCGGCATTG | *REV1* |
| REV1-R | TGTGCAACCATTCGTTCCTTGA | *REV1* |
| REV3_upstr_F | CGAGTGCAGTGCGTCTAGAAATAGTGT | *REV3* |
| REV3_F | GCATGCACACCCCTCATAGTAAGT | *REV3* |
| REV3_2400B | TGGCATTTGACTCTGGCAAGTTCC | *REV3* |
| AS36_SGS1 | CGTGCGTTTCGAAGTGGATTG | *SGS1* |
| JK341_SGS1_int_fwd | TGCAAACTTTGTCGAACGATAC | *SGS1* |
| JK342_SGS1-int_rev | CGACAAGAGAACTAGCCATGTG | *SGS1* |
| ESrsH_F | CAGCTATCCTGATACTACTGCTT | *SRS2* |

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|---|---|---|
| JK300_SRS2_int_fwd | GCTTCCTCAATCACAAGGAAAG | *SRS2* |
| JK301_SRS2_int_rev | TCTAGTCAGCAAACGAAAGGTG | *SRS2* |
| STM1_upstr_F | CAAATTTCTCTTCCCCCCAC | *STM1* |
| STM1_F | GCTGAAAAGGAAGCTCAAGC | *STM1* |
| STM1_R2 | CAACCTTCTTAGCTTCTGGG | *STM1* |
| JK105_TOF1_fwd | TTCTGAAGACCACAGCAACG | *TOF1* |
| TOF1_in_F | CAAGCATACATCTGCAAGACACT | *TOF1* |
| TOF1_in_R | AAGTTACCATCCTATCCTGCTCA | *TOF1* |
| KU70_test_SP | TTAATTGACTCTCGGTAGCCAAGTT | *YKU70* |
| KU70_int_F | GCATGAAGATATCAGACAAGAAGC | *YKU70* |
| KU70_int_R | TGCTCGATGAACGGAACC | *YKU70* |
| Hyg-R | GACTGTCAAGGAGGGTATTC | |
| JK183_hygRleft_rev | ACAGTCACATCATGCCCCTG | |
| HIS3H_R | CCTGTGTGGACGTTAATCACTTGCGAT | |
| For amplification of *RAD27*::*rad27-HphMX4* fragments | | |
| RAD27_ASP | AAAAACTGGCAAAAAAAGAGAAGTATTAGATGAAAAAAGTTCGTGTATACAAATATCTATGTTACATATACATAGGCCACTAGTGGATCTG | |
| RAD27_G240D_mut | TTGCATAATGCTTGGTTGTGACTACTGTGAAAGCATCAGAGGTGTTGATCCAGTGACAGCCTTAAAATTGATA | |
| RAD27_R104A_R105A_K130A_R127A | AGCGGTCTTCAGCTGCTGTGGAAACAGAAAAAAAACTGGCAGAGGCAACAACAGAATTGGAAAAGATGAAGCAAGAAAGAGCTTTGGTGGCTGTCTCAAA | |
| RAD27_G67S_mut | ACGGTGGGCAGTTGACCAATGAAGCCGGTGAAACAACGTCACACTTGATGTCCATGTTTTATAGGACACTGA | |

**Table S3.** Yeast strains used in this project

| Strain | Genotype | Notes |
|---|---|---|
| SMYU | *MATa, leu2-Δ1, trp1-Δ63, ura3-52, his3-200, ade2Δ::KanMX4, HAP1-wt, ChrIII(75594-75641)::*$P_{Gal1}$*-UR-*$(GAA)_{124}$*-A3-TRP1* | (1) |
| SMYU7/8 | SMYU *mre11::HphMX4* | |
| SMYU14/16 | SMYU *msh3::HphMX4* | |
| SMYU22/23 | SMYU *rad52::HphMX4* | |
| SMYU28/31/32 | SMYU *msh6::HphMX4* | |
| SMYU29/41 | SMYU *rad51::HphMX4* | |
| SMYU30/89 | SMYU *msh2::HphMX4* | |
| SMYU35/36 | SMYU *rad59::HphMX4* | |
| SMYU39/40 | SMYU *srs2::HphMX4* | |
| SMYU44/46 | SMYU *rad27::HphMX4* | |
| SMYU42/43 | SMYU *tof1::HphMX4* | |
| SMYU47/48 | SMYU *stm1::HphMX4* | |
| SMYU51/52 | SMYU *mus81::HphMX4* | |
| SMYU55/67 | SMYU *yku70::HphMX4* | |
| SMYU60/61 | SMYU *rad6::HIS3* | |
| SMYU63/64 | SMYU *pol32::HphMX4* | |
| SMYU70/291 | SMYU *rad5::HphMX4* | |
| SMYU71/72 | SMYU *rad27::rad27-4A-HphMX4* | |
| SMYU73/73 | SMYU *sgs2::HphMX4* | |
| SMYU76/77 | SMYU *rad18::HIS3* | |
| SMYU81/82 | SMYU71 *rad5::HIS3* | |
| SMYU83/84/85 | SMYU71 *yku70::HIS3* | |
| SMYU86/87 | SMYU71 *tof1::HIS3* | |
| SMYU90/93 | SMYU *rev3::HIS3* | |
| SMYU95/96 | SMYU *chl1::HIS3* | |
| SMYU101/102 | SMYU *rad27::rad27-G67S-HphMX4* | |
| SMYU114/120 | SMYU *rad27::rad27-G240D-HphMX4* | |
| SMYU116/117 | SMYU *mlh1:: HphMX4* | |
| SMYU125/127 | SMYU *rev1::HIS3* | |
| SMYU129/131 | SMYU *rad30::HIS3* | |
| SMYU133/134 | SMYU *ogg1::HphMX4* | |
| SMYU135/136 | SMYU *rad2::HphMX4* | |
| SMYU137/138 | SMYU *rad14::HphMX4* | |
| SMYU143/144 | SMYU *exo1::HIS3* | |
| SMYU150 | SMYU114 *tof1::HIS3* | |
| SMYU151 | SMYU120 *tof1::HIS3* | |
| SMYU152/152 | SMYU *apn1::HIS3* | |
| SMYU157/162 | SMYU114 *rad6::HIS3* | |
| SMYU191/192 | SMYU *pol3-D520V* | |
| SMYU195/196/197/198 | *SMYU rfa1-S351P* | |

| | | |
|---|---|---|
| SMYU201/202 | SMYU *dna2-H547A* | |
| SMYU204/208 | SMYU63 *rev1::HIS3* | |
| SMYU220/221/222 | SMYU *pms1::HphMX4* | |
| SMYU225/226 | SMYU129 *rev3::HphMX4* | |
| SMYU227 | SMYU131 *rev3::HphMX4* | |
| SMYU233/234 | SMYU *rfa1-K45E* | |
| SMYU244/245 | SMYU *mlh2::HIS3* | |
| SMYU255/256 | SMYU *mlh3::HIS3* | |
| SMYU298/299 | SMYU *rev1-CD* | |
| SMYUI2/4 | *MATa, leu2-Δ1, trp1-Δ63, ura3-52, his3-200, ade2Δ::KanMX4, HAP1-wt, ChrIII(75594-75641)::A3-(GAA)*$_{124}$*-UR-P*$_{Gal1}$*-TRP1* | |
| SMYUI10/11 | SMYUI2 *rev1::HIS3* | |
| SMYUI15/16 | SMYUI *yku70::HIS3* | |
| SMYUI22/23 | SMYUI2 *pol32::HphMX4* | |
| SMYUI30/31 | SMYUI2 *rad52:: HphMX4* | |
| SMYUI34/35/36 | SMYUI2 *chl1::HIS3* | |
| SMYUD14/15/16 | *MATa, leu2-Δ1, trp1-Δ63, ura3-52, his3-200, ade2Δ::KanMX4, HAP1-wt, ChrIII(75594-75641)::P*$_{Gal1}$*-UR-(GAGAA)*$_{74}$*GA-A3-TRP1* | |
| SMYUID13/14/15 | *MATa, leu2-Δ1, trp1-Δ63, ura3-52, his3-200, ade2Δ::KanMX4, HAP1-wt, ChrIII(75594-75641)::A3-(GAGAA)*$_{74}$*GA-UR-P*$_{Gal1}$*-TRP1* | |
| SMYUD23/24/25 | *MATa, leu2-Δ1, trp1-Δ63, ura3-52, his3-200, ade2Δ::KanMX4, HAP1-wt, ChrIII(75594-75641)::P*$_{Gal1}$*-UR-(GAGAAGAAA)*$_{41}$*GAG-A3-TRP1* | |
| SMYUID16/17 | *MATa, leu2-Δ1, trp1-Δ63, ura3-52, his3-200, ade2Δ::KanMX4, HAP1-wt, ChrIII(75594-75641)::A3-(GAAGAAGAAA)*$_{41}$*GAG-UR-P*$_{Gal1}$*-TRP1* | |
| SMYUD21/22 | *MATa, leu2-Δ1, trp1-Δ63, ura3-52, his3-200, ade2Δ::KanMX4, HAP1-wt, ChrIII(75594-75641)::P*$_{Gal1}$*-UR-(GAGAAGGAAA)*$_{37}$*GA-A3-TRP1* | |
| SMYUID25/26 | *MATa, leu2-Δ1, trp1-Δ63, ura3-52, his3-200, ade2Δ::KanMX4, HAP1-wt, ChrIII(75594-75641)::A3-(GAAGAAGGAAA)*$_{37}$*GA-UR-P*$_{Gal1}$*-TRP1* | |
| SMYUD4/5/6 | *MATa, leu2-Δ1, trp1-Δ63, ura3-52, his3-200, ade2Δ::KanMX4, HAP1-wt, ChrIII(75594-75641)::P*$_{Gal1}$*-UR-(GAAGAA)*$_{64}$*-A3-TRP1* | |
| SMYUID1/2 | *MATa, leu2-Δ1, trp1-Δ63, ura3-52, his3-200, ade2Δ::KanMX4, HAP1-wt, ChrIII(75594-75641)::A3-(GAAGAA)*$_{64}$*-UR-P*$_{Gal1}$*-TRP1* | |
| SMYP1/3 | *MATa, leu2-Δ1, trp1-Δ63, ura3-52, his3-200, ChrIII(75594-75641)::P*$_{Gal1}$*-UR-GAA*$_{124}$*-A3-TRP1* | |
| SMYP4/7 | *MATa, leu2-Δ1, trp1-Δ63, ura3-52, his3-200, pol1-Y868F, ChrIII(75594-75641)::P*$_{Gal1}$*-UR-(GAA)*$_{124}$*-A3-TRP1* | (3) |

| | | |
|---|---|---|
| SMYP7/15 | *MATa, leu2-Δ1, trp1-Δ63, ura3-52, his3-200, pol1-Y869A, ChrIII(75594-75641)::*$P_{Gal1}$*-UR-*$(GAA)_{124}$*-A3-TRP1* | (3) |
| SMYPI9 | *MATa, leu2-Δ1, trp1-Δ63, ura3-52, his3-200, ChrIII(75594-75641)::A3-*$(GAA)_{124}$*-UR-*$P_{Gal1}$*-TRP1* | |
| SMYPI4/5/6 | *MATa, leu2-Δ1, trp1-Δ63, ura3-52, his3-200, pol1-Y869A, ChrIII(75594-75641)::A3-*$(GAA)_{124}$*-UR-*$P_{Gal1}$*-TRP1* | (3) |

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