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Supplementary Materials for

Mutation at a distance caused by homopolymeric guanine repeats in *Saccharomyces cerevisiae*

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Other Supplementary Material for this manuscript includes the following:
(available at advances.sciencemag.org/cgi/content/full/2/5/e1501033/DC1)

- table S1 (Microsoft Excel format). Summary table of 318 sequenced *ura3* mutants from G_0, G_{14}-*ORF*, and G_{14}-*repeat* strains.

Supplementary Materials

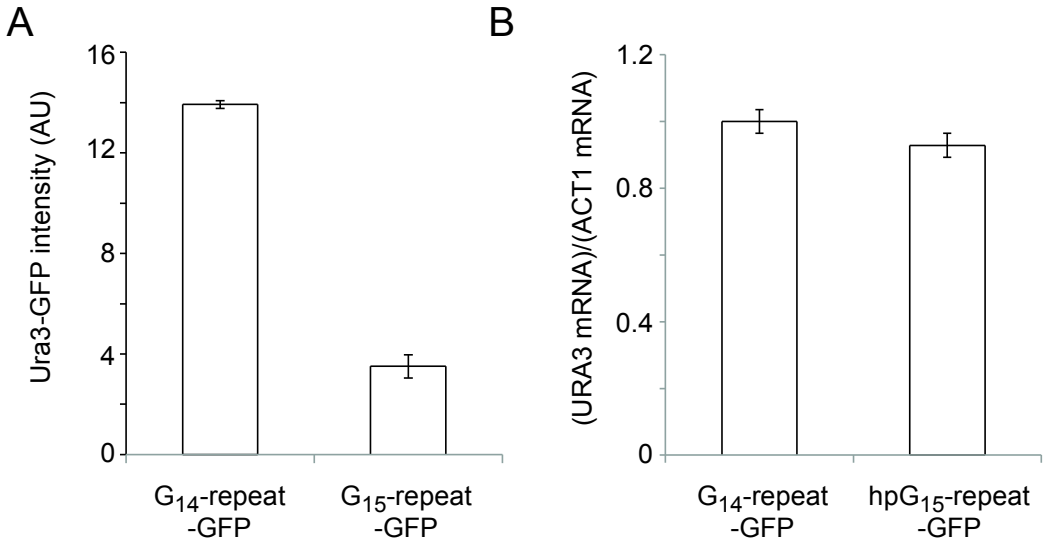

fig. S1. Expansion of the polyguanine repeat (*G14* to *G15*) reduces the Ura3 protein abundance but not the mRNA level. (**A**) The *URA3* gene was tagged with GFP in the *G14-repeat* and *G15-repeat* strains. Ura3-GFP intensity was used to represent the protein level and measured using the fluorescence activated cell sorter. (**B**) mRNA levels were measured using quantitative PCR.

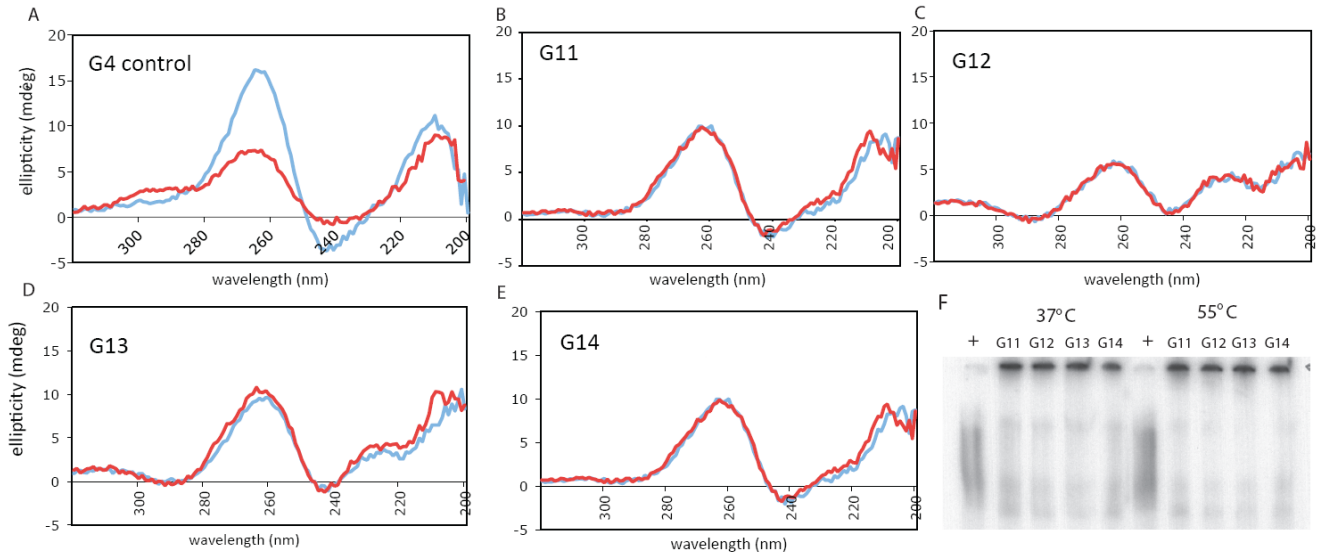

fig. S2. *G11* to *G14* sequences do not stop DNA polymerase from synthesizing DNA, whereas G-quadruplex does. *(***A-E**), We analyzed the structures formed by *G11*, *G12*, *G13* and *G14* oligos incubated in the presence of two ions, K^+ and Na^+. Incubating potential G-quadruplex forming oligos in the presence of either Na^+ or K^+ ions leads to the formation of different structures which can be detected by circular dichroism. K^+ is the preferred ion and

leads to conformationally distinct, stable structures with higher peaks [66, 67]. The peaks observed for the control G-quadruplex structure showed a distinct increase in stability in the presence of K^+ ions compared to Na^+, recapitulating the results of previous work using this same G-quadruplex [50]. However, the $G_{11\text{-}14}$ sequences all showed a lesser peak than the control G-quadruplex, showing no consistent differences between different lengths of G (G_{11} formed just as high a peak as G_{14}), and K+ ions did not induce a different or more stable structure compared to Na+ ions. These combined results suggest that G-quadruplexes are not the causative agent of G_{13+} induced mutagenesis. F, DNA polymerase stop assays were performed on templates containing either a known G-quadruplex forming sequence or homopolymeric G repeats of 11-14 nucleotides. The G-quadruplex forming sequence acts a positive control (lanes labeled “+”), showing that G-quadruplex formation blocks DNA synthesis in this assay. The templates containing $G_{11\text{-}14}$ were synthesized across, supporting that these sequences do not form G-quadruplex structures. The assay was carried out at 37°C and 55°C to test for potential heat lability of structures.

table S1. Summary table of 318 sequenced *ura3* mutants from G_0, G_{14}-*ORF*, and G_{14}-*Repeat* strains.

table S2. Chromosome insertion position and replication timing for engineered G_{14}-*URA* inserts.

Gene	Chr.	Position	Trep (min)	Direction
YLR090W	XII	320701→322080	38	against replication
YLR093C	XII	326513→327415	36	with replication
YLR098C	XII	337527→339473	32	with replication
YLR108C	XII	366667→368124	18	against replication
YOLO25W	XV	274957→276939	12	with replication
YORO32C	XV	389771→391075	36	against replication