

Supplemental Table 1. Expanded clones from YJK146 (CAG)₁₄₀ sequenced for repeat integrity.

Clone	Repeats added	Forward repeats sequenced	Reverse repeats sequenced	Flanking forward ~330bp	Flanking reverse ~420bp
1	73	107	127	+	+
2	95	148	95	+	+
3	27	113	109	+	+
4	93	174	135	+	+
5	142	111	147	+	+
6	88	81	140	+	+
7	70	96	113	+	+
8	73	82	139	+	+
9	145	104	117	+	+
10	59	77	125	+	+
11	91	138	182	+	+
12	75	168	179	+	+
13	77	167	148	+	+
14	94	171	142	+	+
15	101	169	158	+	+
16	98	194	185	+	+
17	62	170	146	+	+
18	70	137	114	+	+
19	98	173	141	+	+
20	92	175	133	+	+
21	88	174	131	+	+

Supplemental Table 2. Non-selective instability of YJK146 (CAG)₁₄₀ strain.

<i>Strain media</i>	Total PCRs	Expansions		Contractions	
		#	%	#	%
<i>WT</i> glucose	95	1	1.1	5	5.3
<i>WT</i> galactose	143	1	0.7	25	17.5

3.3-fold difference in contraction frequency for (CAG)₁₄₀ grown on glucose compared to galactose; Fisher's exact test p-value 0.0153

Supplemental Table 3. Primers used in this study.

Name	Sequence	Notes
JK109_CAG_NcoI	agctccatggGCCGCCAGTGTGATGGATAGC	CAG cloning
JK110_CAG_NotI	agctgcggccgcGGCTTTTGCAAAAAGCTCTGCAGATC	CAG cloning
FlankL	CCTTTGCCATTTATTGTCGC	CAG sequencing
CanF	TGCTTCTCCTCTATGTCGGC	CAG sequencing
JK161_tetbal_fwd	agctccatggcctaggGCTACTTGGAGCCACTATCGAC	CAG spacer, no repeat cloning
JK162_T50_rev	agctccatggcctaggGCTACTTGGAGCCACTATCGAC	CAG spacer cloning
JK163_T100_rev	agctccatggtaccGGACGGGTGTGGTCGCCATGAT	CAG spacer cloning
JK164_T150_rev	agctccatggtaccTGTGGCGCCGGTGATGCCGGCC	CAG spacer cloning
JK165_T340_rev	agctgcatgcggtaccCATCGGTCGACGCTCTCCCT	No repeat cloning
TrpS-F	TCGATTTCTGACTGGGTTGGAAG	Verify ChrIII integration
36a-R	TAGCATACGTGTAATGATAGACGATTTTC	Verify ChrIII integration
A36b-F	ACGTGTACAGTTCTCTTTACATCATC	Verify ChrIII integration
JK153_CAN140_fwd	GTGATGGATAGCAGCAGCAG	CAG repeat length
JK171_CAG140_rev	GCATGCGGCTTTTGCAAAAAGCTC	CAG repeat length
JK316_CAG140_fwd	TAGTGGCTCCAAGTAGCCCTAG	CAG repeat length
JK317_CAG140_rev	GTAGTAGGTTGAGGCCGTTGAG	CAG repeat length
JK318_CAG140_fwd	CCGCACTGCTCCGAACAATC	CAG repeat length
JK319_CAG140_rev	GAAGCAGCCCAGTAGTAGGTTG	CAG repeat length
JK167_CAN1_fwd	GGCCGCTCTAGAAGTAGTGGAT	CAN1 sequencing
JK168_CAN1_rev	CTCTTGGAACGGATTTTCTGG	CAN1 sequencing
JK169_CAN1_fwd	GTTTTAGCCATTATCGGGTTTC	CAN1 sequencing
JK170_CAN1_rev	AAGCTGCCTTTGTGTGCTTAAT	CAN1 sequencing
ACT1 F	CTCCACCACTGCTGAAAGAG	ChIP qPCR
ACT1 R	GTGATGACTTGACCATCTGG	ChIP qPCR
5' UAS 3 F	CGGAAGAGCATTGACAAATATTGA	ChIP qPCR
5' UAS 3 R	GGAAATAACAATGTCTGCGAGTG	ChIP qPCR
T150 1900 F	TAGTGGATCCCCGGGCTGC	ChIP qPCR
T150 2100 R	CTCGAGGGGGGGCCCTCTCC	ChIP qPCR

* Primers for generating and confirming gene knockouts are available upon request.

Supplemental Table 4. Strains used in this study.

Strain	Genotype	Background	Comments
CH1585	<i>MATa, leu2-Δ1, trp1-Δ63, ura3-52, his3-200</i>		Shishkin <i>et al</i> , 2009
KS	<i>bar1::HIS3, can1::KanMX</i>	CH1585	Shah <i>et al</i> , 2014
YJK146	<i>ChrIII(75594-75641)::UAS_{GAL}-(CAG)₁₄₀-P_{GAL}-CAN1-TRP1</i>	KS	Made with Swal cut pYes3-G4G1C1-Fori-CAG140-tetbal1-rev
YJK154	<i>ChrIII(75594-75641)::UAS_{GAL}-TET340-P_{GAL}-CAN1-TRP1</i>	KS	Made with Swal cut pYes3-G4G1C1-Fori-tet340
YJK156	<i>ChrIII(75594-75641)::UAS_{GAL}-(CAG)₉₃-P_{GAL}-CAN1-TRP1</i>	KS	Made with Swal cut pYes3-G4G1C1-Fori-CAG93-tetbal2-rev
YJK159	<i>srs2::HphMX4</i>	YJK146	
YJK161	<i>rad5::HphMX4</i>	YJK146	
YJK162	<i>rad52::HphMX4</i>	YJK146	
YJK189	<i>sgs1::HphMX4</i>	YJK146	
YJK190	<i>rad51::HphMX4</i>	YJK146	
YJK191	<i>msh2::HphMX4</i>	YJK146	
YJK192	<i>msh3::HphMX4</i>	YJK146	
YJK193	<i>msh6::HphMX4</i>	YJK146	
YJK194	<i>pol32::HphMX4</i>	YJK146	
YJK196	<i>pif1::HphMX4</i>	YJK146	
YJK198	<i>pif1-m2</i>	YJK146	Made using pVS31 (Schulz and Zakian, 1994)
YJK199	<i>pol30-89-LEU2</i>	YJK146	Made using pJH2480 (Lydeard <i>et al</i> , 2010)
YJK200	<i>mre11::HphMX4</i>	YJK146	
YJK202	<i>mus81::HphMX4</i>	YJK146	
YJK221	<i>rev3Δ</i>	YJK146	Made using pAM56 (Holbeck and Strathern, 1997)
YJK228	<i>yen1::NatMX4</i>	YJK146	
YJK229	<i>mus81::HphMX4, yen1::NatMX4</i>	YJK202	

1. Shishkin, A. A. *et al*. Large-Scale Expansions of Friedreich's Ataxia GAA Repeats in Yeast. *Molecular Cell* **35**, 82–92 (2009).
2. Shah, K. A., McGinty, R. J., Egorova, V. I. & Mirkin, S. M. Coupling Transcriptional State to Large-Scale Repeat Expansions in Yeast. *Cell Reports* **9**, 1594–1602 (2014).
3. Schulz, V. P. & Zakian, V. A. The *Saccharomyces* PIF1 DNA helicase inhibits telomere elongation and de novo telomere formation. *Cell* **76**, 145–155 (1994).

4. Lydeard, J. R. *et al.* Break-induced replication requires all essential DNA replication factors except those specific for pre-RC assembly. *Genes & Development* **24**, 1133–1144 (2010).
5. Holbeck, S. L. & Strathern, J. N. A role for REV3 in mutagenesis during double-strand break repair in *Saccharomyces cerevisiae*. *Genetics* **147**, 1017–1024 (1997).

