Table S1. Yeast strain list

Description	Number	Genotype
Parent BIR strain[1]	LSY3881	*MATa-inc lys2::LEU2 hml::oripRS hmr::ampR AVT2::lys-HOcs::KanMX6 ade3::GAL-HO PTK1::TRP1-ys2* (Chr XI 70 kb donor)
BIR strain + ITS	LSY3944	*MATa-inc lys2::LEU2 hml::oripRS hmr::ampR AVT2::lys-HOcs::KanMX6 ade3::GAL-HO PTK1::TRP1-ys2 ITS-URA3*
BIR strain - ITS	LSY3945	*MATa-inc lys2::LEU2 hml::oripRS hmr::ampR AVT2::lys-HOcs::KanMX6 ade3::GAL-HO PTK1::TRP1-ys2 URA3*
BIR strain + ITS (48 kb)	LSY4096	*MATa-inc lys2::LEU2 hml::oripRS hmr::ampR AVT2::lys-HOcs::KanMX6 ade3::GAL-HO PTK1::TRP1-ys2 ITS-URA3 (48 kb)*
BIR strain - ITS (48 kb)	LSY4097	*MATa-inc lys2::LEU2 hml::oripRS hmr::ampR AVT2::lys-HOcs::KanMX6 ade3::GAL-HO PTK1::TRP1-ys2 URA3 (48 kb)*
pif1-m2	W3819-4C	*MATα pif1-m2*
***pif1-m2* + ITS**	LSY3989-42	*MATa-inc lys2::LEU2 hml::oripRS hmr::ampR AVT2::lys-HOcs::KanMX6 ade3::GAL-HO PTK1::TRP1-ys2 ITS-URA3 pif1-m2*
***pif1-m2* - ITS**	LSY3989-52	*MATa-inc lys2::LEU2 hml::oripRS hmr::ampR AVT2::lys-HOcs::KanMX6 ade3::GAL-HO PTK1::TRP1-ys2 URA3 pif1-m2*
***mph1*Δ + ITS**	LSY4146	*MATa-inc lys2::LEU2 hml::oripRS hmr::ampR AVT2::lys-HOcs::KanMX6 ade3::GAL-HO PTK1::TRP1-ys2 ITS-URA3 mph1::HphMX*
***mph1*Δ - ITS**	LSY4145	*MATa-inc lys2::LEU2 hml::oripRS hmr::ampR AVT2::lys-HOcs::KanMX6 ade3::GAL-HO PTK1::TRP1-ys2 URA3 mph1::HphMX*
***sgs1*Δ + ITS**	LSY4628	*MATa-inc lys2::LEU2 hml::oripRS hmr::ampR AVT2::lys-HOcs::KanMX6 ade3::GAL-HO PTK1::TRP1-ys2 ITS-URA3 sgs1::HphMX*
***tlc1*Δ + ITS**	LSY4155	*MATa-inc lys2::LEU2 hml::oripRS hmr::ampR AVT2::lys-HOcs::KanMX6 ade3::GAL-HO PTK1::TRP1-ys2 ITS-URA3 tlc1::HphMX*
***tlc1*Δ - ITS**	LSY4156	*MATa-inc lys2::LEU2 hml::oripRS hmr::ampR AVT2::lys-HOcs::KanMX6 ade3::GAL-HO PTK1::TRP1-ys2 URA3 tlc1::HphMX*
TetO-TLC1	yT529	*MATα ADE2 tlc::HIS3MX6-PrTetO2-TLC1 cdc10::CDC10-mCherry-KanMX*
***TetO-TLC1* + ITS**	LSY4629-23C	*MATa-inc lys2::LEU2 hml::oripRS hmr::ampR AVT2::lys-HOcs::KanMX6 ade3::GAL-HO PTK1::TRP1-ys2 ITS-URA3 tlc1::HIS3MX6-Pr-TetO2-TLC1*
BIR strain with Y' element	LSY4318	*MATa-inc lys2::LEU2 hml::oripRS hmr::ampR AVT2::lys-HOcs::KanMX6 ade3::GAL-HO PTK1::TRP1-ys2 Y'element4-URA3*
BIR strain with $(GT)_{32}$	LSY4425	*MATa-inc lys2::LEU2 hml::oripRS hmr::ampR AVT2::lys-HOcs::KanMX6 ade3::GAL-HO PTK1::TRP1-ys2 $(GT)_{32}$-URA3*
***pol32*Δ + ITS**	LSY4314	*MATa-inc lys2::LEU2 hml::oripRS hmr::ampR AVT2::lys-HOcs::KanMX6 ade3::GAL-HO PTK1::TRP1-ys2 ITS-URA3*

		p*ol32::HphMX*
***pol32Δ* - ITS**	LSY4315	*MATa-inc lys2::LEU2 hml::oripRS hmr::ampR AVT2::lys-HOcs::KanMX6 ade3::GAL-HO PTK1::TRP1-ys2 URA3*, *pol32::HphMX*
***pol32Δ* + $(GT)_{32}$**	LSY4452	*MATa-inc lys2::LEU2 hml::oripRS hmr::ampR AVT2::lys-HOcs::KanMX6 ade3::GAL-HO PTK1::TRP1-ys2 $(GT)_{32}$-URA3 pol32::HphMX*
***pol32Δ* + Y' element**	LSY4453	*MATa-inc lys2::LEU2 hml::oripRS hmr::ampR AVT2::lys-HOcs::KanMX6 ade3::GAL-HO PTK1::TRP1-ys2 Y' element-URA3 pol32::HphMX*
***pif1-m2* + $(GT)_{32}$**	LSY4427	*MATa-inc lys2::LEU2 hml::oripRS hmr::ampR AVT2::lys-HOcs::KanMX6 ade3::GAL-HO PTK1::TRP1-ys2 $(GT)_{32}$-URA3 pif1-m2*
***pif1-m2* + Y' element**	LSY4428	*MATa-inc lys2::LEU2 hml::oripRS hmr::ampR AVT2::lys-HOcs::KanMX6 ade3::GAL-HO PTK1::TRP1-ys2 Y' element-URA3 pif1-m2*
***pif1-m2*, *tlc1Δ* +ITS**	LSY4426	*MATa-inc lys2::LEU2 hml::oripRS hmr::ampR AVT2::lys-HOcs::KanMX6 ade3::GAL-HO PTK1::TRP1-ys2 ITS-URA3 tlc1::HphMX pif1-m2*
***rad10Δ* + ITS**	LSY4203	*MATa-inc lys2::LEU2 hml::oripRS hmr::ampR AVT2::lys-HOcs::KanMX6 ade3::GAL-HO PTK1::TRP1-ys2 ITS-URA3 rad10::HphMX*
***rad10Δ* – ITS**	LSY4202	*MATa-inc lys2::LEU2 hml::oripRS hmr::ampR AVT2::lys-HOcs::KanMX6 ade3::GAL-HO PTK1::TRP1-ys2 URA3 rad10::HphMX*

[1]All strains are in the W303 background (*leu2-3,112 trp1-1 can1 ura3-1 ade2-1 his3-11,15 RAD5*), only differences from this genotype are listed above.

Table S2. Oligonucleotides

F to insert ITS 2.5	GCTACGTGATTACATTACCATGTGTGGGTGTGTGGGTGTGTGGGTGTGTGGGTGTGTGGGTGT GTGGGTGTGTGGGTGTGTGGGGTGCAGGTCGACAACCCTTAAT
R to insert ITS 2.5	AAATCAGTATCAGTGGGGACGCAGCGTACGGATATCACCTA
F to insert *URA3* only 2.5	GCTACGTGATTACATTACCAGTGCAGGTCGACAACCCTTAAT
R extend homology 2.5	TGGTCTTAGACTTTGGTAACTCTTCCAGTTCCTTTTTCAAAAGATCTTTCAAATCAGTATCAGTGG GGAC
F extend homology 2.5	GACAAAGTAGATGATGGGTTAGTCAATATGGCTACACTAAACATCACTGAGCTACGTGATTACAT TACCA
F insert 2.5	CAAGATTCATTGACGTCGACAC
R insert 2.5	CCGTCATAGTCGAACCAAAATC
F to insert Y' element	GCTACGTGATTACATTACCAGCGTTCCATGACGAGCGCTGGACTGCAGGGTCCGCAGTACGTC AAGCTGCAGTTTAGCAGGCATCATCGACAGTTGAGGTGCAGGTCGACAACCCTTAAT
F to insert $(GT)_{32}$	GCTACGTGATTACATTACCATGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGT GTGTGTGTGTGTGTGTGTGTGTGCAGGTCGACAACCCTTAAT
F to insert ITS 48	CGCGCTCAATAACTTCCTCGTGTGTGGGTGTGTGGGTGTGTGGGTGTGTGGGTGTGTGGGTGT GTGGGTGTGTGGGTGTGTGGGTGCAGGTCGACAACCCTTAAT
R to insert ITS 48	ATGAAGACTATTCGCCGTACGCAGCGTACGGATATCACCTA
F to insert *URA3* only 48	CGCGCTCAATAACTTCCTCGTGCAGGTCGACAACCCTTAAT
F extend homology 48	TCCTGGTGGAGAATAATGGTAGGCTTGGCGGCTCTGAGATGGAGAAATAGTTTCAAGCCGCGC GCTCAATAACTTCCTCG
R extend homology 48	GCAGCACCTGCGTCATTCACGCTGAAGCGGCAGCAAGCATTTTCGATCAGCTCCAATTAAATGA AGACTATTCGCCGTAC
F screen insert 48	GTAAAGGATAGTACCATAGGAG
R screen insert 48	GCAATTTACTCCACTAGACC
R Y' truncations	CACTTTAGATGGGGTAACTCC

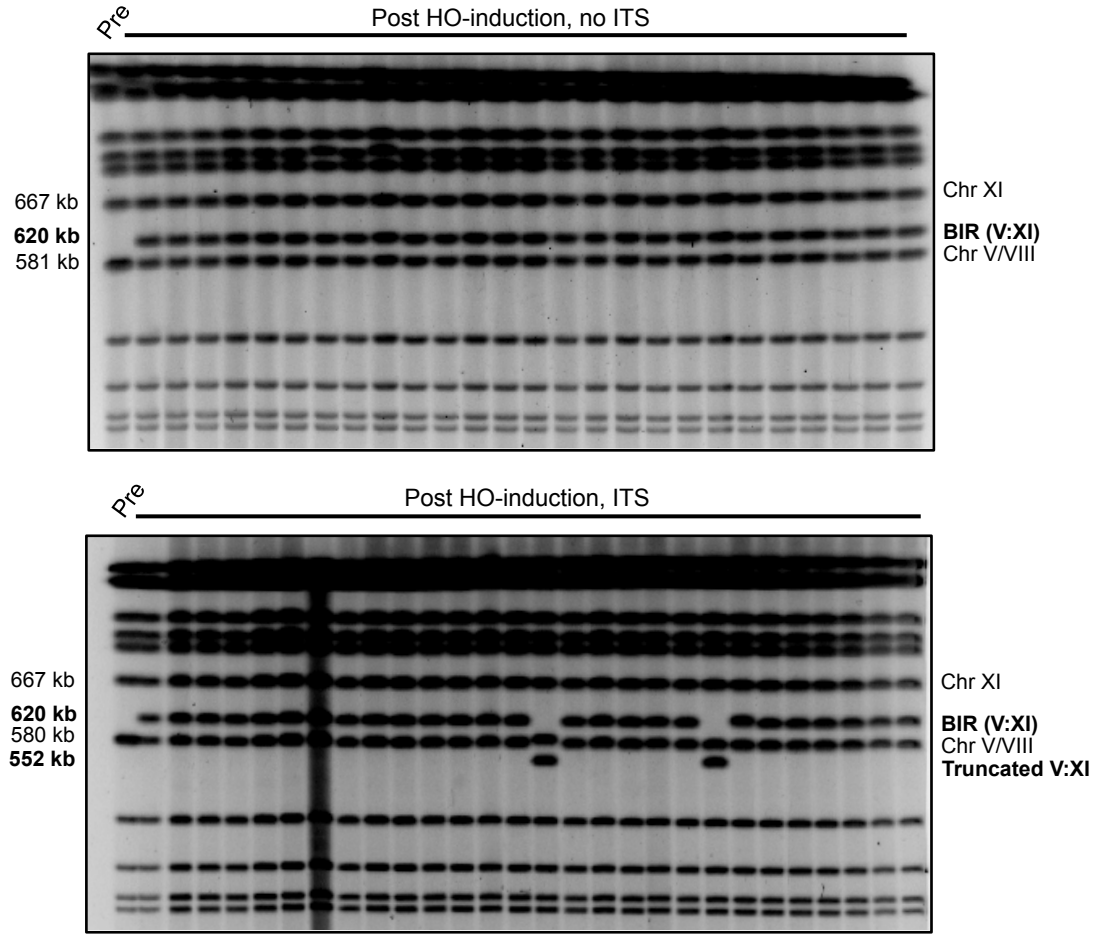


Figure S1. Identification of truncated chromosomes by PFGE. Representative pulsed-field gels showing chromosome sizes pre-HO induction and of independent clones after HO induction strains without or with the ITS.

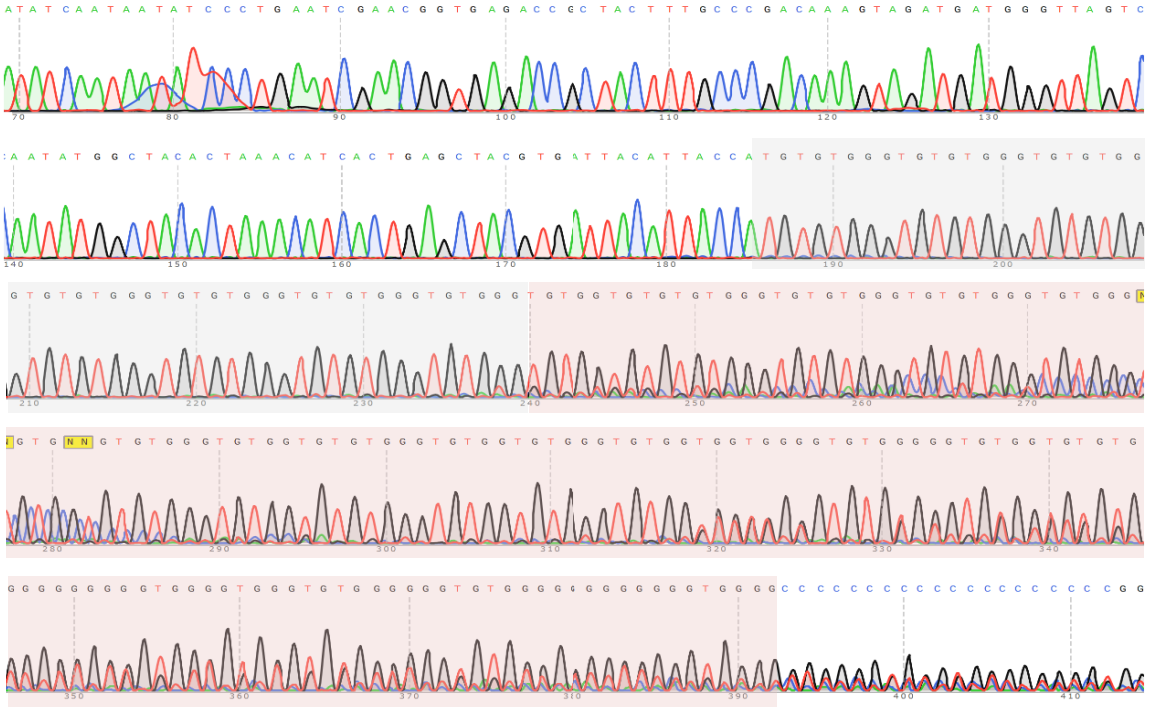
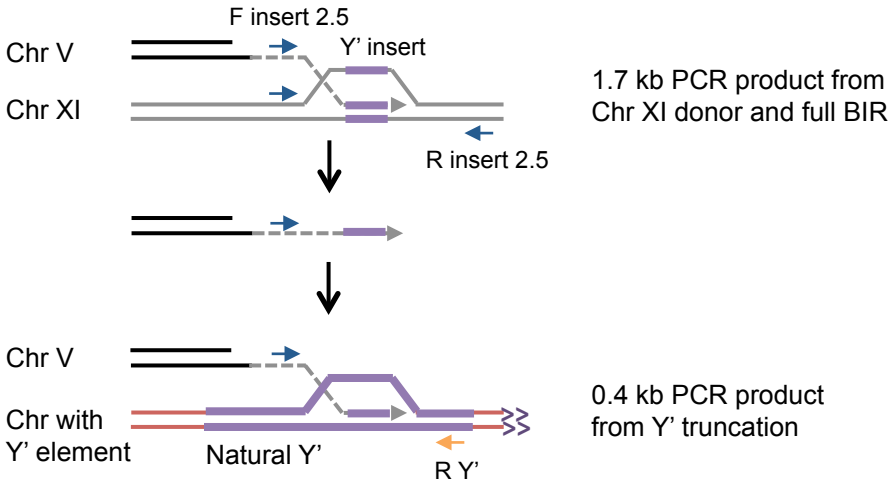


Figure S2. Ectopic telomere formation at the ITS. Example trace from sequencing one of the truncated chromosome products. Sequences of the inserted ITS are highlighted in grey, and telomeric sequences added by the cell are highlighted in red. The short string of cytosines at the end is an artifact of the C-tailing used to PCR amplify the region for sequencing.



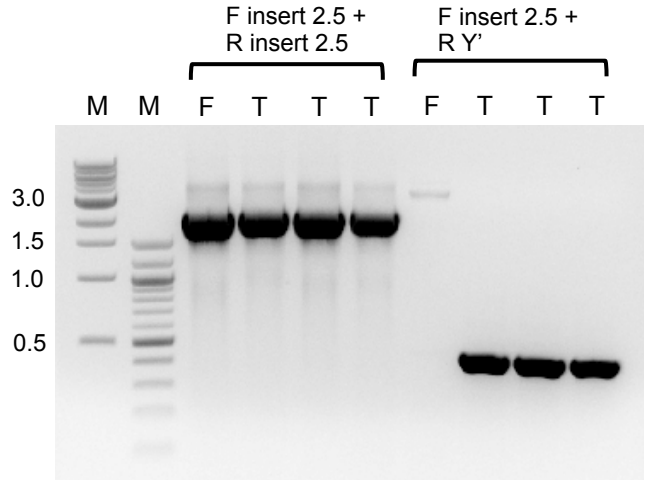


Figure S3. Y' truncated chromosomes result from HR. DNA extracted from Full BIR (F) or Truncated BIR (T) products was PCR amplified with F insert 2.5 and R insert 2.5 primers, which amplify the Y' insert and *URA3* present on the Chr XI donor and Full BIR product, or with F insert 2.5 + R Y' primers to detect truncations resulting from HR between the Y' insert and full-length Y' element present at many yeast telomeres. The image shows 3 truncations of 10 total tested by PCR. M refers to 1 kb and 0.1 kb size markers.