Table S1. Background stains used to integrate TNR tracts.

Strain No.	Genotype	Derived from strain	Source
FY86	*MAT*a *ura3-52 leu2Δ1 his3Δ200*		Winston, 1995
JSY1501	*MAT*a *ura3-52 leu2Δ1 his3Δ200 msh3Δ::KANMX*	FY86	Kantartzis, 2012
JSY1515	*MAT*a *ura3-52 leu2Δ1 his3Δ200 msh3Δ::hisG*	FY86	This study

Table S2. Initial expansion size of trinucleotide repeat tracts[a]

Genotype	Tract sequence	Median size (net change)[b]	Range (net change)[b]
MSH3 (n=61)	*CAG*	42 (+17)	31-67 (+6 to +42)
MSH3 (n=89)	*CTG*	33 (+8)	26-43 (+1 to +18)
msh3Δ (n=38)	*CAG*	31 (+6)	31-48 (+6 to +16)
msh3Δ (n=35)	*CTG*	33.5 (+8)	28-39 (+3 to +14)

[a] Initial expansion sizes obtained from all time course experiments.

[b] Number of repeats in TNR tract

Supplementary Figures

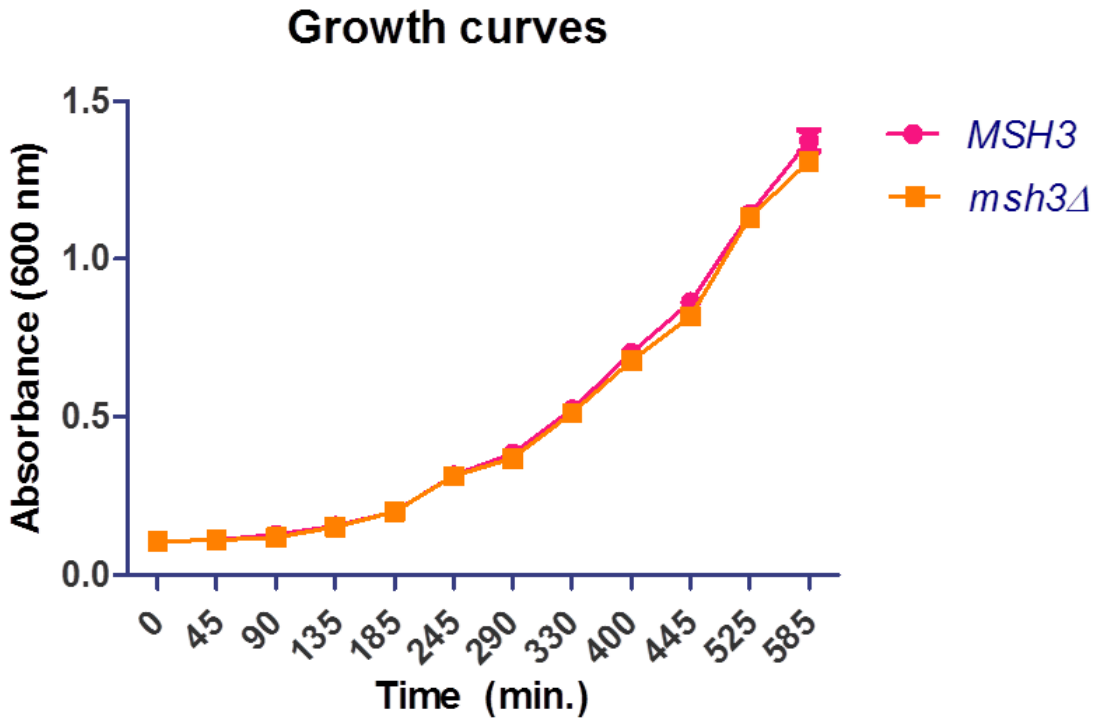


Figure S1. Growth curves of *MSH3* and *msh3Δ* strains. Growth curve experiments were performed with two independent isolates each of *MSH3(CTG)*$_{25}$ and *MSH3(CAG)*$_{25}$, in parallel, measuring absorbance (600 nm) as a function of time. These curves were averaged and the standard error of the mean (S.E.M.) calculated for each time point (pink circles). Similarly, growth curve experiments were performed with two independent isolates each of *msh3Δ(CTG)*$_{25}$ and *msh3Δ(CAG)*$_{25}$ (orange squares).

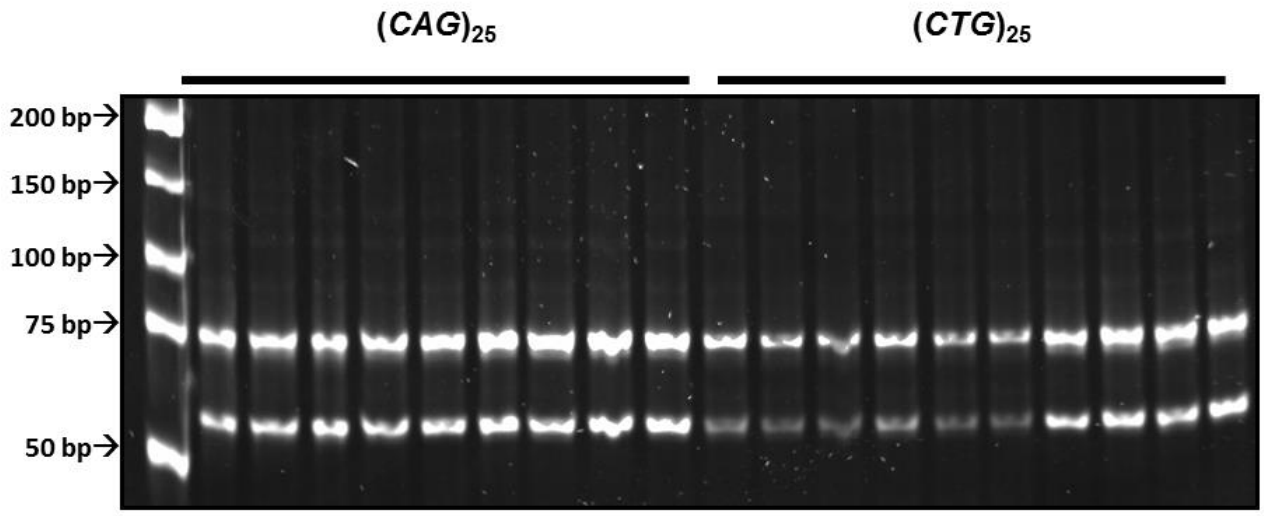


Figure S2. Control PCR reactions to amplify unexpanded TNR tract from plasmid.
Nineteen independent PCR amplification of the TNR tract from pBL170 $(CAG)_{25}$ and pBL169 $(CTG)_{25}$ (Miret *et al.*, 1998) separated by native gel electrophoresis on a 12% polyacrylamide gel. The PCR products were digested with *Sph*I and *Afl*II, which releases a product containing only the tract. The lower band is a product of this digestion. The unexpanded tract is 75 bp (25 repeats).

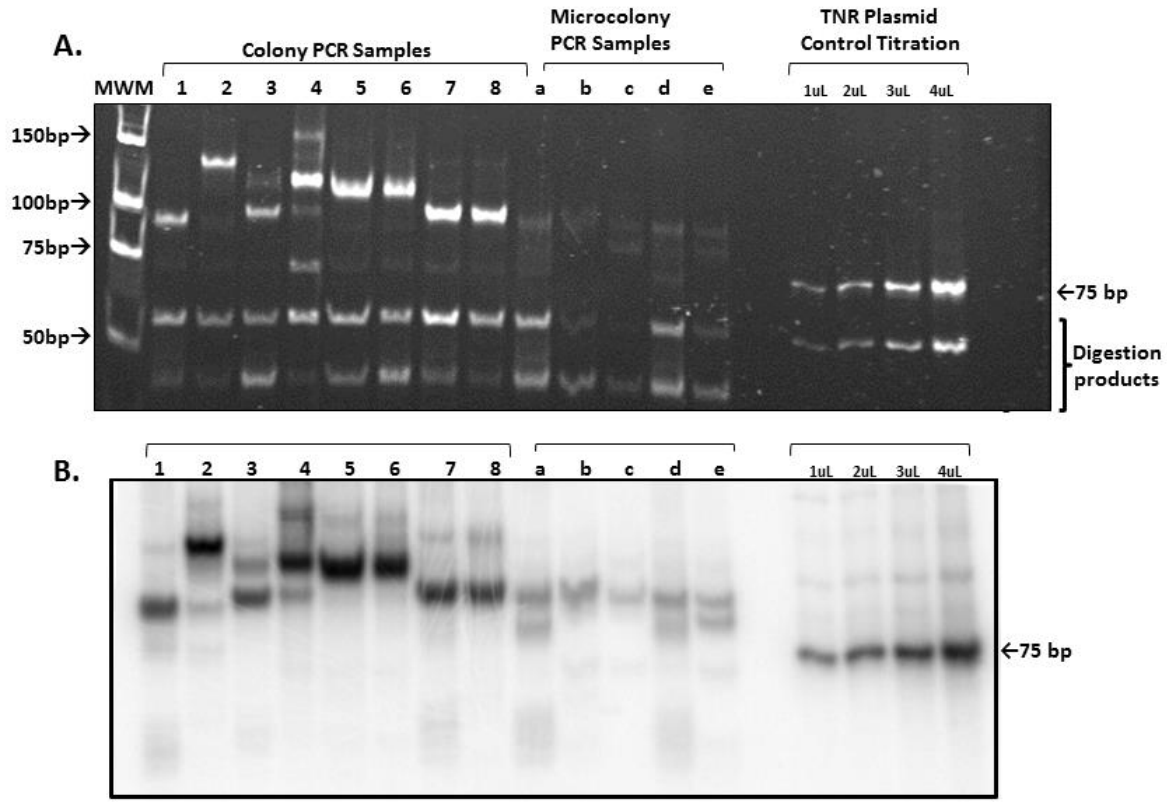


Figure S3. Specificity of probe used for Southern blots. The TNR tract from *MSH3* was amplified by PCR from representative colonies and microcolonies and digested with *Sph*I and *Afl*II to release the TNR tract. **A.** The resulting products were separated on a 12% polyacrylamide gel. A titration of a control reaction using plasmid DNA as a template was electrophoresed alongside these reactions (1μL – 4μL). **B.** The gel in **A** was subjected to Southern blot using a (*CTG*)-containing probe. The TNR probe specifically interacted with TNR-containing fragments, but not other DNA products.

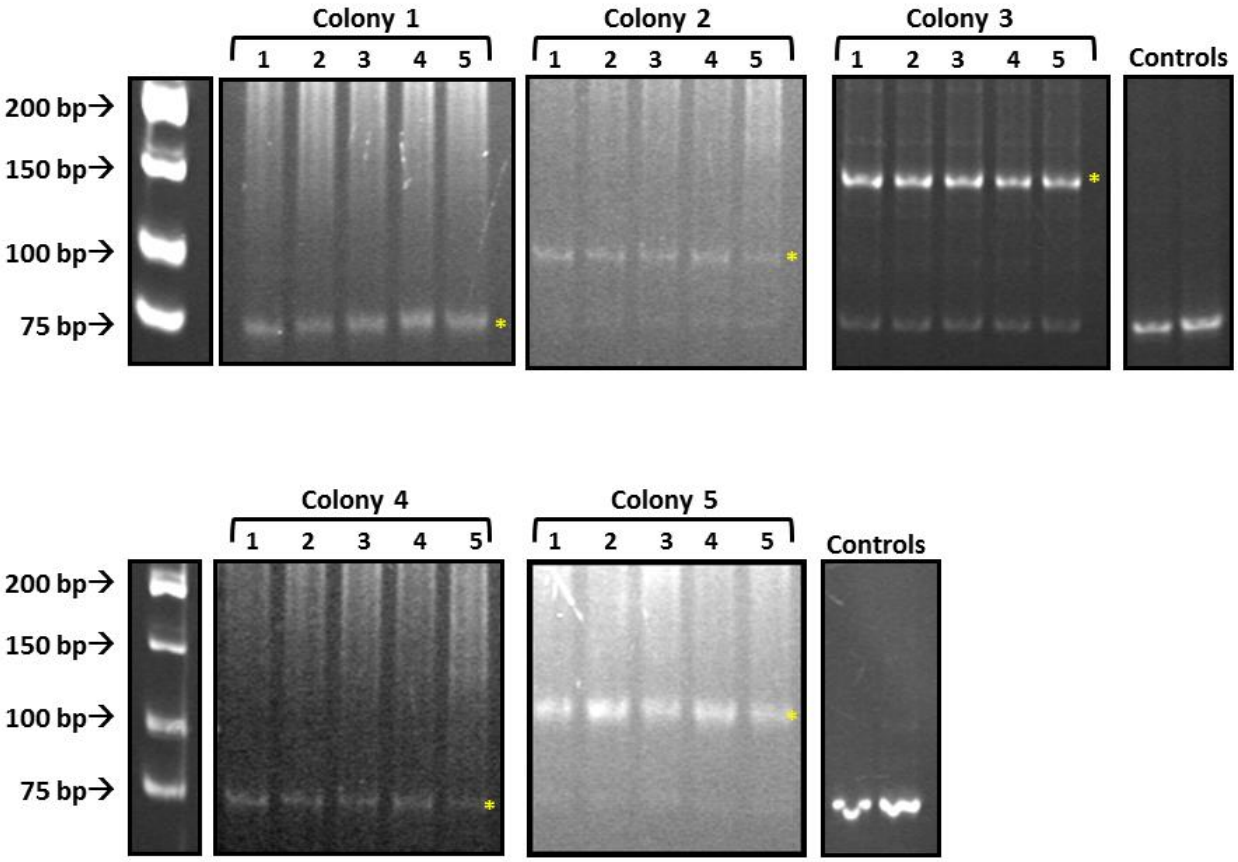


Figure S4. Control colony PCR amplification of unexpanded and expanded TNR tracts. In each panel, five independent PCR reactions were performed in parallel from a single colony selected on 5-FOA to determine reproducibility of these reactions. PCR products were digested with *Sph*I and *Afl*II to release the tract. The other digestion products are not shown. The unexpanded tract is 75 bp.

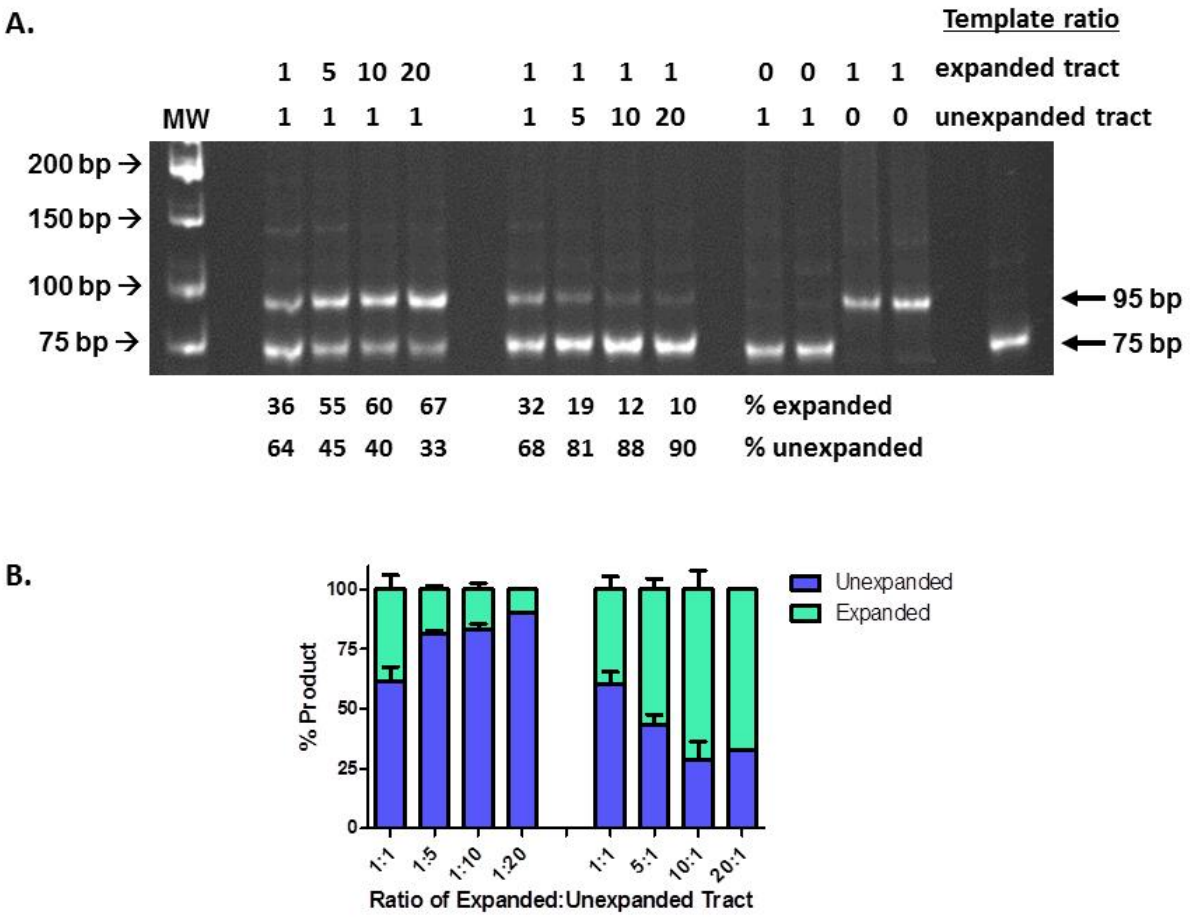


Figure S5. PCR amplification products correlate with input template DNA. A. Different ratios of unexpanded:expanded (1:1, 1:5, 1:10, 1:20) tract (genomic DNA) were used as a substrate for PCR; the total template DNA was constant at 5 μg. The amount of input template correlates with the PCR product output, although there is a small but significant bias toward the unexpanded tract. **B.** The relative amounts of expanded and unexpanded PCR product as a function of input template ratio is plotted. The error bars indicate the S.E.M. of three independent experiments.

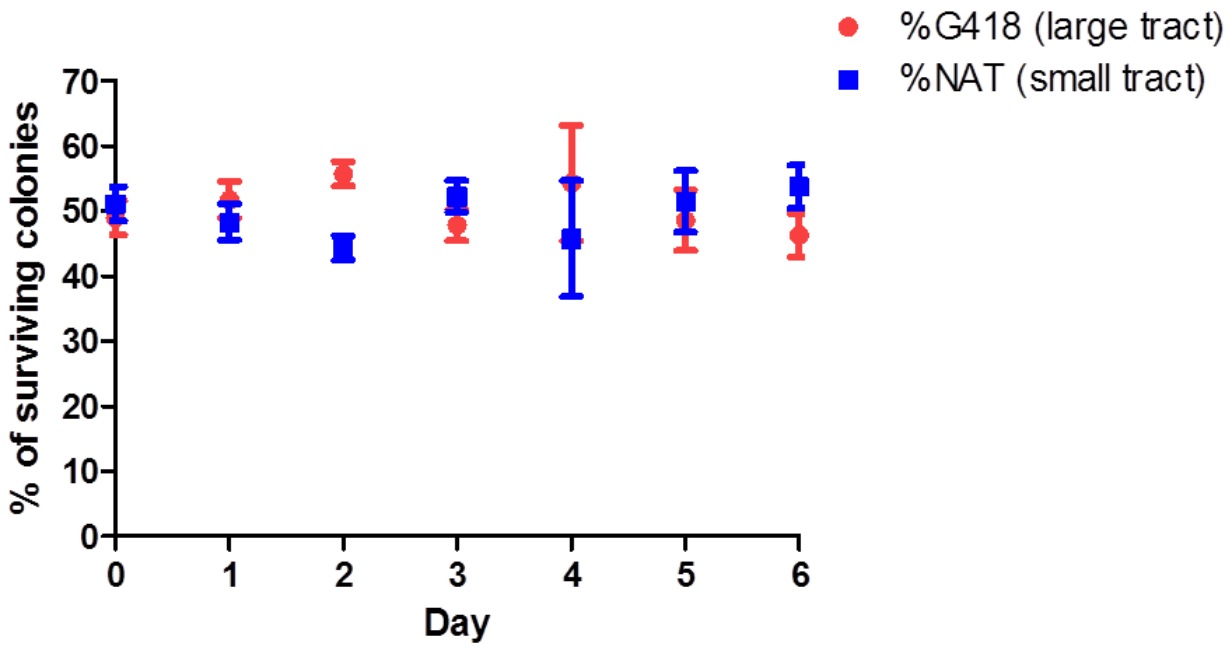
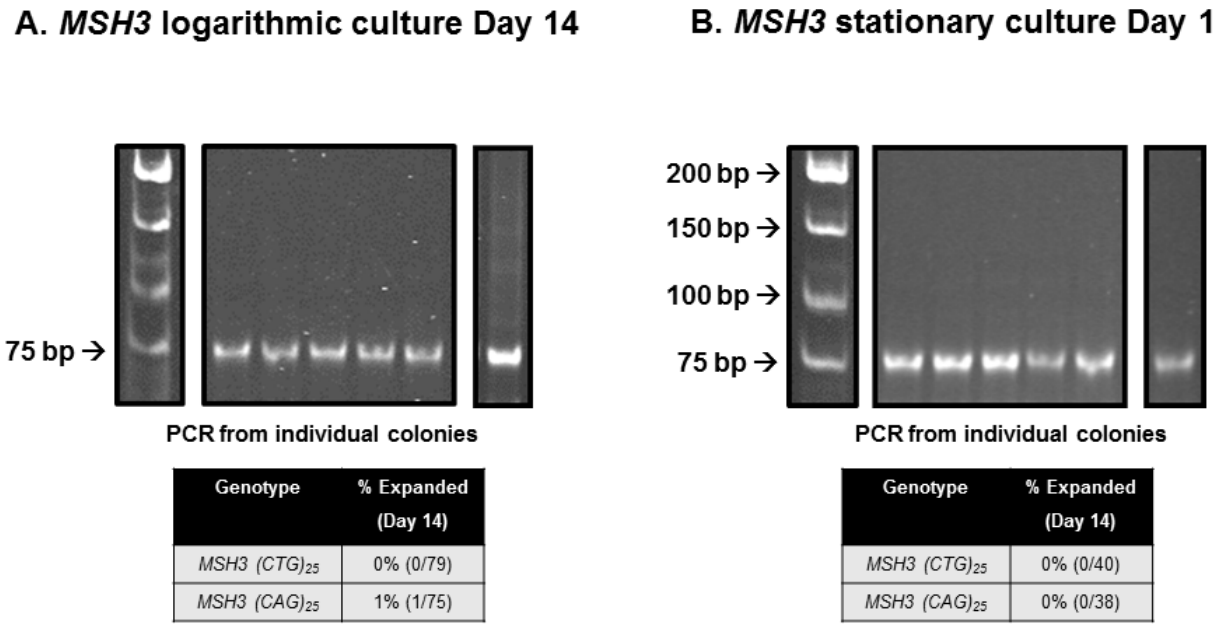


Figure S6. A larger TNR tract does not confer a selective advantage. A *MSH3* strain encoding a large TNR tract (>40 repeats) was transformed with a low copy plasmid encoding the KANMX cassette, conferring resistance to G418. A *MSH3* strain encoding a short (<30 repeats) TNR tract was transformed with a low copy plasmid encoding the NATMX cassette, conferring resistance to clonNAT. These cells were mixed at a 1:1 ratio and the mixture was grown as a log phase culture (see **Fig. S4A**), in the absence of selection, for 6 days. Cells were plated and the percentage that retained either the KANMX (red circles) or the NATMX (blue squares) cassette was determined. An average of three independent experiments (± SEM) is shown.



Genotype	% Expanded (Day 14)
MSH3 $(CTG)_{25}$	0% (0/79)
MSH3 $(CAG)_{25}$	1% (1/75)

Genotype	% Expanded (Day 14)
MSH3 $(CTG)_{25}$	0% (0/40)
MSH3 $(CAG)_{25}$	0% (0/38)

Figure S7. Liquid time course experiments in *MSH3(CTG)$_{25}$* and *MSH3(CAG)$_{25}$* logarithmic and stationary cultures to determine population tract dynamics. We performed time course experiments with *MSH3* strains containing *unexpanded CTG* and *CAG* tracts in **A.** logarithmic and **B.** stationary phase cultures. Tract sizes were confirmed by PCR prior to starting the experiment. **Top panel:** Samples from Day 14 cultures were plated on minimal media lacking histidine to obtain individual colonies. Colony PCR was performed to amplify the TNR tract from colonies to determine individual tract lengths within the population. The arrows indicate tracts that have incurred an additional expansion. The number below

each tract indicates the number of repeats within each tract. Control PCR reactions using the TNR plasmid (C) were performed alongside each set as a marker for the 75 base pair (bp) tract (25 repeats). Summary of expansion frequency at Day 14 in the different genetic backgrounds tested, based on PCR amplification of tracts from individual colonies. Mutation rates from these data were calculated and are shown in **Table 1**.

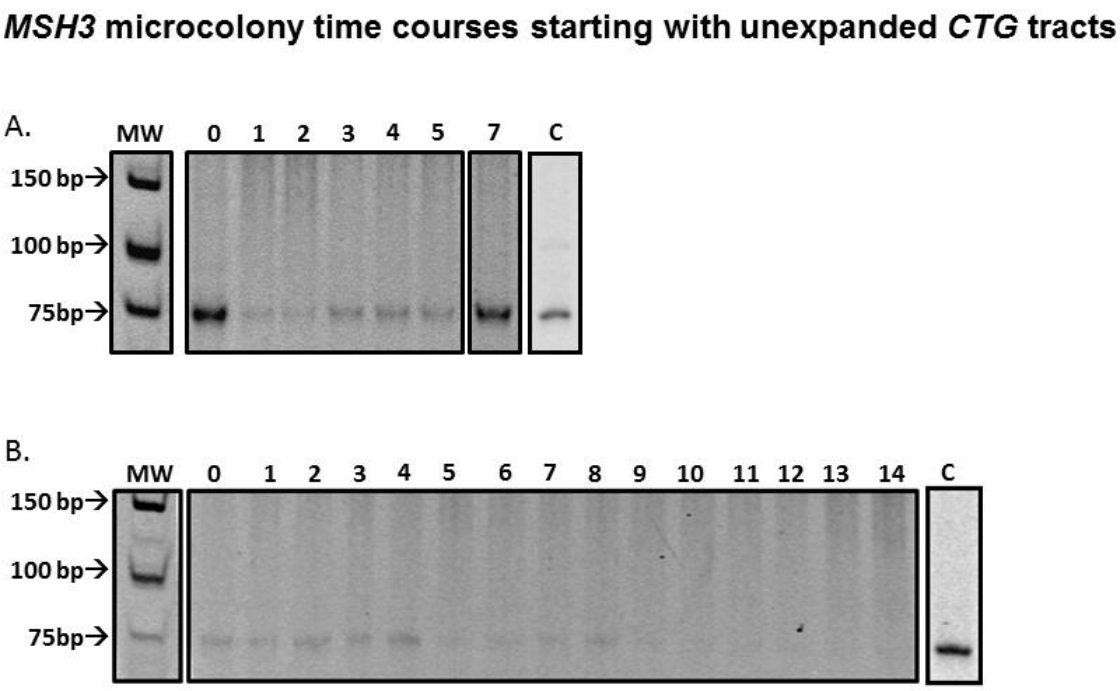


Figure S8. Unexpanded TNR tracts are stable starting from a single cell. Two examples of microcolony time courses starting with *unexpanded* tracts in a *MSH3* $(CTG)_{25}$ background. Tract sizes were confirmed by PCR. Individual cells from these colonies were isolated and allowed to undergo 8-10 rounds of replication, resulting in a microcolony approximately 250-1000 cells in size. A single cell was then taken from this microcolony to propagate another microcolony. The remainder was used to amplify the TNR tract by PCR to determine tract length (see **Fig. 10** for cartoon). No tract changes were observed. Both gels are 12% polyacrylamide gels stained with EtBr; the images of been inverted for ease of viewing. The lanes marked **C** in each panel indicate the 75bp tract amplified from the TNR plasmid control.

The numbers across the top of the gels indicate the time point. A mutation rate for these experiments was calculated and is shown in **Table 1**.