

# Additional file 1

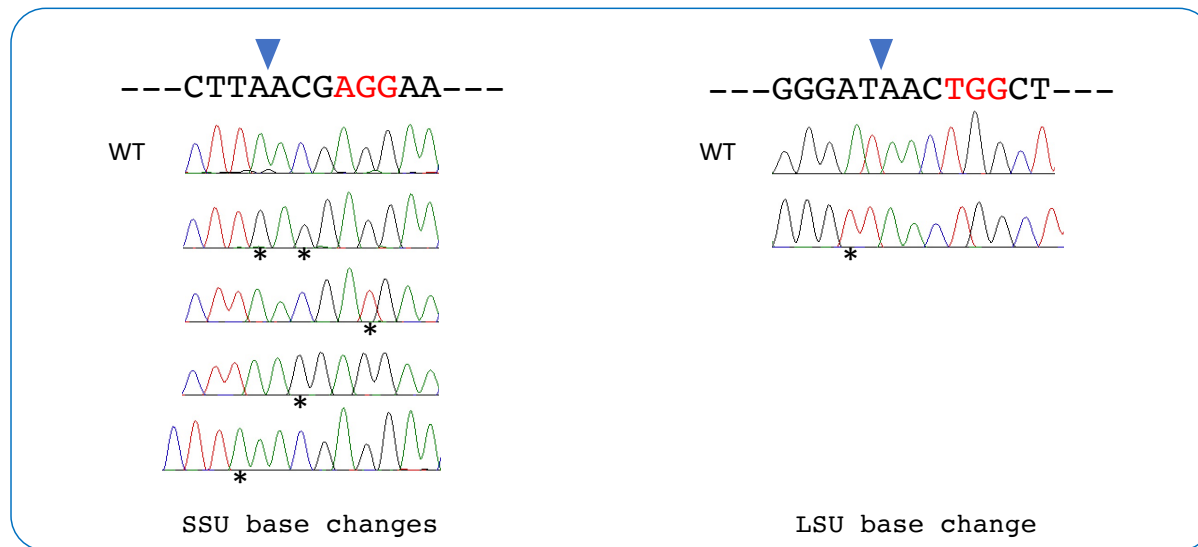
## qPCR data elaboration

Efficiency from both repeats and all samples																																																							
SSU primers	Act primers	Strain	Ct SSU	Avg Ct SSU	Ct ACT	Avg Ct ACT	Delta SSU YJ0-SS2	Delta ACT YJ0-SS2	E_ssu^Dssu	E_act^Dact	Ratio SS2/YJ0	Delta SSU YJ0-LS7	Delta ACT YJ0-LS7	E_ssu^Dssu	E_act^Dact	Ratio LS7/YJ0																																							
1,54	1,45	YJ0	17,85		29,73																																																		
1,58	1,49		17,96	17,70333333	29,38	29,48	-4,216666667	-3,276666667	0,157555901	0,282980733	0,556772538	-3,89	-2,89	0,181806621	0,328437625	0,553549919																																							
1,56	1,49		17,3		29,33																																																		
1,55	1,4																																																						
1,54	1,42			21,81		32,82																																																	
1,53	1,44		SS2	21,9	21,92	32,72	32,75666667																																																
1,55	1,42			22,05		32,73																																																	
1,53	1,45																																																						
1,52	1,45		LS7	21,36		32,27																																																	
1,55	1,49			21,66	21,59333333	32,58	32,37																																																
1,57	1,49	21,76			32,26																																																		
1,58	1,51																																																						
1,55	1,47	YJ0	17,47		29,2																																																		
1,57	1,47		17,63	17,26666667	29,05	29,07	-3,566666667	-2,143333333	0,20948372	0,437908302	0,478373482	-2,116666667	-0,686666667	0,395486113	0,767553689	0,515255309																																							
1,54	1,48		16,7		28,96																																																		
1,59	1,51																																																						
1,59	1,52			20,49		31,41																																																	
1,55	1,49		SS2	20,86	20,83333333	31,15	31,21333333																																																
1,555	1,468888889			21,15		31,08																																																	
Avg E ssu	Avg E act																																																						
0,02093407	0,034108631		LS7	19,18		29,81																																																	
SDV ssu	SDV act			19,5	19,38333333	29,96	29,75666667																																																
		19,47			29,5																																																		
		<table border="1"> <thead> <tr> <th>Mutant/YJ0</th> <th>Ratios</th> <th>SS2 AVG</th> <th>LS7 AVG</th> <th>Global AVG</th> <th>SDV</th> <th>AVG - SDV</th> <th>AVG + SDV</th> </tr> </thead> <tbody> <tr> <td>repeat one</td> <td>SS2/YJ0</td> <td>0,556772538</td> <td>0,51757301</td> <td>0,534402614</td> <td>0,525987812</td> <td>0,0369219</td> <td>0,489065912</td> </tr> <tr> <td>repeat two</td> <td>LS7/YJ0</td> <td>0,553549919</td> <td></td> <td></td> <td></td> <td></td> <td></td> </tr> <tr> <td></td> <td>SS2/YJ0</td> <td>0,478373482</td> <td></td> <td></td> <td></td> <td></td> <td></td> </tr> <tr> <td></td> <td>LS7/YJ0</td> <td>0,515255309</td> <td></td> <td></td> <td></td> <td></td> <td></td> </tr> </tbody> </table>														Mutant/YJ0	Ratios	SS2 AVG	LS7 AVG	Global AVG	SDV	AVG - SDV	AVG + SDV	repeat one	SS2/YJ0	0,556772538	0,51757301	0,534402614	0,525987812	0,0369219	0,489065912	repeat two	LS7/YJ0	0,553549919							SS2/YJ0	0,478373482							LS7/YJ0	0,515255309					
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Based on: Nucleic Acids Res. 2001 May 1; 29(9): e45. doi: 10.1093/nar/29.9.e45																																																							
Mutant/YJ0 rDNA copies ratio = $\frac{1.55^{(YJ0\ SSU\ ct - Mutant\ SSU\ ct)}}{1.47^{(YJ0\ ACT\ ct - Mutant\ ACT\ ct)}}$																																																							

## Additional File 2

### Point mutations generated though CRISPR are homogenized throughout the rDNA

Individual base-pair mutations generated by NHEJ were obtained by transforming strain YJ0 only with a pCAS plasmid engineered to target either position 534 in the 18S or position 2818 in the 25S gene (Fig. 1). Several clones had the same mutation and the total number of mutant base configurations we detected was small (four around 18S position 534 and one around 25S position 2818), not surprising since each surviving mutation needs to produce a functional, even if suboptimal, rRNA. Most base-pair change mutants grew more slowly than the parent. Overall, almost all transformants fulfilled the expectation that any repeat with a wild type sequence around the target site would not survive Cas9 attack. However, some transformants had no change around the target site, and actually grew at wild type rates or faster. We did not analyze those further.



**Figure 1.** Individual base pair changes around the Cas9 cut sites. Only sense-strand sequences are shown, 5' ends on the left. PAM sequences are marked red. Arrowheads point to Cas9 cut sites. The wild type sequence at each site is indicated above the chromatograms of representative yeast transformants bearing base changes (asterisks) produced through NHEJ. The traces show no peak heterogeneity among the sequenced PCR fragments at the mutated sites, suggesting that the same base change spread to all rDNA repeats.