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## **Supplemental Information**

# **Ribonucleotides Are Signals for Mismatch Repair**

#### of Leading-Strand Replication Errors

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Figure S1. Quantifying ribonucleotide incorporation using alkaline-hydrolysis data, Related to Figure 1. Curves are derived from strains with wild type polymerases (black), the L612M Pol  $\delta$  mutator allele (green), or the M644G Pol  $\epsilon$  mutator allele using data from one of four independent experiments. Panels A-H refer to lagging strand data (Probe A in Fig. 1C). Panels I and J refer to

leading strand data (Probe B in Fig. 1C). (A) Fluorescence intensity curves for one representative alkaline-hydrolysis experiment with  $RNH201^+$  strains (Fig. 1C, Probe A, RNH201 = +). (B) As per panel A, but for  $rnh201\Delta$  strains (Figure 1C, Probe A, RNH201 = -). (C) An exponential transformation (constructed from ladder positions) is applied to convert gel migration distances from panel A into fragment sizes. (D) As per panel C, but for starting values from panel B. (E) Total intensities in each fragment size bin (panel C) are converted to average intensities per bin by dividing by bin width. The results are normalized to average fragment fractions by dividing by total lane intensity. (F) As per panel E, but with starting values from panel D. (G) Least squares regression of panel E data versus the sum of two Weibull distributions. The area under each curve yields the fraction of fragments in high- and low- mobility peaks. (H) As per panel G, but versus curves from panel F. (I) As per panel E, but with Probe B (leading strand). Analyses were performed as in panels A-H. (J) As per panel F, but with Probe B (leading strand). When comparing panels F and J, note that the fraction of fragments in the high mobility (Fig.1C) peaks track with *in vitro* ribonucleotide incorporation rates (Fig. 1A).

	pol2	-M644G	mutation	95%	5-FOA <sup>R</sup>	no URA3	URA3	Trar	nsitions	Fram	neshifts
m	sh3,(	6∆/msh3∆	rate (x10 <sup>-7</sup> )	confidence	mutants	change	mutations	#	rate	#	rate
	01	msh3∆	1.6	(1.1-2.5)	122	15	109	9	0.12	50	0.66
-	NH2(	msh3,6∆	110	(88-140)	173	16	166	67	43	56	36
tion	RI	correction fact	tors						360x		54x
URA3 orienta	14	msh3∆	11.0	(8.8-14)	137	17	120	19	1.5	26	2.1
	h20	msh3,6∆	250	(170-350)	164	18	154	64	98	67	100
	rn	correction fact	tors						64x		49x
2	RNF	H201/rnh201∆ c	orrection facto			5.6		1.1			
	p-va	lues							≤ 0.001		0.430
	01	msh3∆	2.90	(2.2-3.9)	125	17	108	4	0.093	88	2.0
2	NH2	msh3,6∆	280	(220-370)	158	16	144	34	60	80	140
tion	RI	correction fact	tors						650x		69x
enta	14	msh3∆	15	(11-21)	139	21	118	3	0.32	33	3.6
8 orie	h20	msh3,6∆	280	(210-360)	122	22	102	30	69	54	120
IRA3	uл	correction fact	tors						210x		35x
C	RNH	H201/rnh201∆ c	correction facto	or ratios					3.1		2.0
	p-values 0								0.10		0.015

Table S1. *pol2-M644G msh3* mutation rates in two *URA3* orientations ± *MSH6* and/or *RNH201*, Related to Figure 2

For comparison, all rates are listed by strain in Table S6. All rates are multiplied by  $10^7$ . C.f. stands for correction factor. Correction factors are compared in Fig. 2A, listed as "*msh3*,6 $\Delta$ /*msh3* $\Delta$ ". Strain origins and related references may be found in Table S5.

Table S2. pol2-M64	44G mutation rates in two URA3 orientations ± MMR and/or RNH201,
<b>Related to Figure 2</b>	

	pol2-M644G	mutation	95%	5-FOA <sup>R</sup>	no URA3	URA3	Trai	nsitions	Fran	neshifts	
ms	sh3,6	⊿/wt MMR	rate (x10 <sup>-7</sup> )	confidence	mutants	change	mutations	#	rate	#	rate
	01	wt MMR	1.7	(1.3-2.3)	342	105	237	24	0.12	12	0.060
<del>.</del>	NH2(	msh3,6∆	110	(88-140)	173	16	166	67	43	56	36
tion	R	correction fact	tors						360x		600x
enta	14	wt MMR	7.6	(4.3-10)	128	4	125	25	1.5	7	0.42
8 orie	h20	msh3,6∆	250	(170-350)	164	18	154	64	98	67	100
IRA3	n	correction fact	tors						66x		250x
2	RNF	<del>1</del> 201/rnh201∆ c	orrection facto	or ratios					5.4		2.4
	p-va	lues							≤ 0.001		0.083
	01	wt MMR	0.83	(0.71-0.96)	246	123	124	36	0.12	14	0.047
2	NH2	msh3,6∆	280	(220-370)	158	16	144	34	60	80	140
tion	R	correction fact	correction factors								3,000x
enta	14	wt MMR	14	(9.8-20)	138	0	138	5	0.51	7	0.71
3 ori	h20	msh3,6∆	280	(210-360)	122	22	102	30	69	54	120
IRA3	r,	correction fact	tors						140x		170x
2	RNF	<del>1</del> 201/rnh201∆ c	correction facto	or ratios				3.7		17	
	p-va	lues							0.058		≤ 0.001
	pol2	-M644G	mutation	95%	5-FOA <sup>R</sup>	no URA3	URA3	Trai	nsitions	Fran	neshifts
m	pol2 sh2/	- <i>M644G</i> 1/wt MMR	mutation rate (x10 <sup>-7</sup> )	95% confidence	5-FOA <sup>R</sup> mutants	no URA3 mutations	URA3 mutations	Trai #	nsitions rate	Fran #	neshifts rate
m	pol2 sh21	<b>-M644G</b> <b>1/wt MMR</b> wt MMR	mutation rate (x10 <sup>-7</sup> ) 1.7	95% confidence (1.3-2.3)	5-FOA <sup>R</sup> mutants 342	no URA3 mutations 105	URA3 mutations 237	Trai # 24	nsitions rate 0.12	Fran # 12	neshifts rate 0.060
m	pol2 sh21	<b>-M644G</b> Δ/wt MMR wt MMR msh2Δ	mutation rate (x10 <sup>-7</sup> ) 1.7 180	95% confidence (1.3-2.3) (120-270)	5-FOA <sup>R</sup> mutants 342 333	no URA3 mutations 105 35	URA3 mutations 237 306	Trai # 24 92	rate 0.12 50	Fran # 12 162	neshifts rate 0.060 88
tion 1 m	pol2 sh21 total	<b>-M644G</b> Δ/wt MMR wt MMR msh2Δ correction fact	mutation rate (x10 <sup>-7</sup> ) 1.7 180 :ors	95% confidence (1.3-2.3) (120-270)	5-FOA <sup>R</sup> mutants 342 333	no URA3 mutations 105 35	URA3 mutations 237 306	Trai # 24 92	rate 0.12 50 420x	Fran # 12 162	neshifts rate 0.060 88 <b>1,500x</b>
entation 1 3	pol2 sh21 ₽2 FNH201 ₽2	<b>-M644G</b> Δ/wt MMR wt MMR msh2Δ correction fact wt MMR	mutation rate (x10 <sup>-7</sup> ) 1.7 180 tors 7.6	95% confidence (1.3-2.3) (120-270) (4.3-10)	5-FOA <sup>R</sup> mutants 342 333 128	no URA3 mutations 105 35 4	URA3 mutations 237 306 125	Trai # 24 92 25	nsitions rate 0.12 50 <b>420x</b> 1.5	Fran # 12 162 7	neshifts rate 0.060 88 <b>1,500x</b> 0.42
3 orientation 1 <b>w</b>	h201∆ RNH201 Si 02 7201∆ RNH201 Si 02	<b>-M644G</b> Δ/wt MMR wt MMR msh2Δ correction fact wt MMR msh3,6Δ	mutation rate (x10 <sup>-7</sup> ) 1.7 180 tors 7.6 250	95% confidence (1.3-2.3) (120-270) (4.3-10) (170-350)	5-FOA <sup>R</sup> mutants 342 333 128 164	no URA3 mutations 105 35 4 18	URA3 mutations 237 306 125 154	Trai # 24 92 25 64	rate 0.12 50 420x 1.5 98	Fran # 12 162 7 67	neshifts rate 0.060 88 <b>1,500x</b> 0.42 100
IRA3 orientation 1	rnh201∆ RNH201 Slod	<b>-M644G</b> Δ/wt MMR wt MMR msh2Δ correction fact wt MMR msh3,6Δ correction fact	mutation rate (x10 <sup>-7</sup> ) 1.7 180 tors 7.6 250 tors	95% confidence (1.3-2.3) (120-270) (4.3-10) (170-350)	5-FOA <sup>R</sup> mutants 342 333 128 164	no URA3 mutations 105 35 4 18	URA3 mutations 237 306 125 154	Tran # 24 92 25 64	nsitions rate 0.12 50 420x 1.5 98 66x	Fran # 12 162 7 67	neshifts rate 0.060 88 <b>1,500x</b> 0.42 100 <b>250x</b>
URA3 orientation 1	pol2 sh21 RNH201 RNH201 RNH201	-M644G Δ/wt MMR wt MMR msh2Δ correction fact wt MMR msh3,6Δ correction fact	mutation rate (x10 <sup>-7</sup> ) 1.7 180 tors 7.6 250 tors correction factor	95% confidence (1.3-2.3) (120-270) (4.3-10) (170-350) >r ratios	5-FOA <sup>R</sup> mutants 342 333 128 164	no URA3 mutations 105 35 4 18	URA3 mutations 237 306 125 154	Trat # 24 92 25 64	rate 0.12 50 420x 1.5 98 66x 6.3	Fran # 12 162 7 67	neshifts rate 0.060 88 <b>1,500x</b> 0.42 100 <b>250x</b> <b>6.0</b>
URA3 orientation 1	pol2 sh21 rwh2017 RNH5017 p-va	-M644G Δ/wt MMR wt MMR msh2Δ correction fact wt MMR msh3,6Δ correction fact 1201/rnh201Δ c	mutation           rate (x10 <sup>-7</sup> )           1.7           180           tors           7.6           250           tors	95% confidence (1.3-2.3) (120-270) (4.3-10) (170-350) >r ratios	5-FOA <sup>R</sup> mutants 342 333 128 164	no URA3 mutations 105 35 4 18	URA3 mutations 237 306 125 154	Tran # 24 92 25 64	nsitions rate 0.12 50 420x 1.5 98 66x 6.3 ≤ 0.001	Fran # 12 162 7 67	neshifts rate 0.060 88 <b>1,500x</b> 0.42 100 <b>250x</b> <b>6.0</b> 0.009
URA3 orientation 1	01 2017 RNH201 2012 2010 2010 2010 2010 2010 2010	-M644G Δ/wt MMR wt MMR msh2Δ correction fact wt MMR msh3,6Δ correction fact lues wt MMR	mutation rate (x10 <sup>-7</sup> ) 1.7 180 tors 7.6 250 tors correction factor	95% confidence (1.3-2.3) (120-270) (4.3-10) (170-350) or ratios (0.71-0.96)	5-FOA <sup>R</sup> mutants 342 333 128 164 246	no URA3 mutations 105 35 4 18 18	URA3 mutations 237 306 125 154 154	Trat # 24 92 25 64 36	rate 0.12 50 420x 1.5 98 66x 6.3 ≤ 0.001 0.12	Fran # 12 162 7 67 14	neshifts rate 0.060 88 <b>1,500x</b> 0.42 100 <b>250x</b> <b>6.0</b> 0.009 0.047
2 URA3 orientation 1 3	NH201 a 2 rnh2014 RNH201 5200	-M644G Δ/wt MMR wt MMR msh2Δ correction fact wt MMR msh3,6Δ correction fact lues wt MMR msh2Δ	mutation           rate (x10 <sup>-7</sup> )           1.7           180           tors           7.6           250           tors           0.83           180	95% confidence (1.3-2.3) (120-270) (4.3-10) (170-350) or ratios (0.71-0.96) (100-220)	5-FOA <sup>R</sup> mutants 342 333 128 164 164 246 254	no URA3 mutations 105 35 4 18 18 123 38	URA3 mutations 237 306 125 154 154 124 224	Tran # 24 92 25 64 	nsitions rate 0.12 50 420x 1.5 98 66x 6.3 ≤ 0.001 0.12 59	Fran # 12 162 7 67 67 14 85	neshifts rate 0.060 88 <b>1,500x</b> 0.42 100 <b>250x</b> <b>6.0</b> 0.009 0.047 60
tion 2 URA3 orientation 1 3	RNH201 d 2010 RNH201 Scion 2010 RNH201 RNH20 RNH200 RNH200 RNH200 RNH200 RNH200 RNH200	-M644G Δ/wt MMR wt MMR msh2Δ correction fact wt MMR msh3,6Δ correction fact ilues wt MMR msh2Δ correction fact	mutation rate (x10 <sup>-7</sup> ) 1.7 180 tors 7.6 250 tors correction factor 0.83 180	95% confidence (1.3-2.3) (120-270) (4.3-10) (170-350) <b>or ratios</b> (0.71-0.96) (100-220)	5-FOA <sup>R</sup> mutants 342 333 128 164 246 254	no URA3 mutations 105 35 4 18 18 123 38	URA3 mutations 237 306 125 154 154 124 224	Trai # 24 92 25 64 36 83	nsitions rate 0.12 50 420x 1.5 98 66x 6.3 ≤ 0.001 0.12 59 480x	Fran # 12 162 7 67 67 14 85	neshifts rate 0.060 88 <b>1,500x</b> 0.42 100 <b>250x</b> <b>6.0</b> 0.009 0.047 60 <b>1,300x</b>
entation 2 URA3 orientation 1 3	1Δ RNH201 d rnh201Δ RNH201 5500 rh201Δ RNH201 7510	-M644G Δ/wt MMR wt MMR msh2Δ correction fact wt MMR msh3,6Δ correction fact lues wt MMR msh2Δ correction fact wt MMR	mutation           rate (x10 <sup>-7</sup> )           1.7           180           tors           7.6           250           tors           0.83           180           :ors           180	95% confidence (1.3-2.3) (120-270) (4.3-10) (170-350) <b>x ratios</b> (0.71-0.96) (100-220) (9.8-20)	5-FOA <sup>R</sup> mutants 342 333 128 164 246 254 254	no URA3 mutations 105 35 4 18 18 123 38 38	URA3 mutations 237 306 125 154 154 124 224 224	Tran # 24 92 25 64 36 83 5	nsitions rate 0.12 50 420x 1.5 98 66x 6.3 ≤ 0.001 0.12 59 480x 0.51	Fran # 12 162 7 67 67 14 85 7	neshifts rate 0.060 88 <b>1,500x</b> 0.42 100 <b>250x</b> <b>6.0</b> 0.009 0.047 60 <b>1,300x</b> 0.71
3 orientation 2 URA3 orientation 1 3	1201∆ RNH201 d x x rnh201∆ RNH201 S 20	<b>-M644G</b> Δ/wt MMR wt MMR msh2Δ correction fact wt MMR msh3,6Δ correction fact ilues wt MMR msh2Δ correction fact wt MMR msh3,6Δ	mutation rate (x10 <sup>-7</sup> ) 1.7 180 tors 7.6 250 tors correction factor 0.83 180 tors 14 280	95% confidence (1.3-2.3) (120-270) (4.3-10) (170-350) r ratios (0.71-0.96) (100-220) (9.8-20) (210-360)	5-FOA <sup>R</sup> mutants 342 333 128 164 246 254 254 138 122	no URA3 mutations 105 35 4 18 18 123 38 38 0 0 22	URA3 mutations 237 306 125 154 154 224 224 224 138 102	Trai # 24 92 25 64 36 83 5 30	nsitions rate 0.12 50 420x 1.5 98 66x 6.3 ≤ 0.001 0.12 59 480x 0.51 69	Fran # 12 162 7 67 67 14 85 7 7 54	neshifts rate 0.060 88 <b>1,500x</b> 0.42 100 <b>250x</b> <b>6.0</b> 0.009 0.047 60 <b>1,300x</b> 0.71 120
IRA3 orientation 2 URA3 orientation 1 3	rmh201Δ RNH201 <sup>d</sup> 3 rmh201Δ RNH201 50 50	-M644G Δ/wt MMR wt MMR msh2Δ correction fact wt MMR msh3,6Δ correction fact lues wt MMR msh2Δ correction fact wt MMR msh3,6Δ correction fact	mutation           rate (x10 <sup>-7</sup> )           1.7           180           tors           7.6           250           tors           .ors           0.83           180           tors           .ors           180           tors	95% confidence (1.3-2.3) (120-270) (4.3-10) (170-350) (170-360) (170-360) (170-360)	5-FOA <sup>R</sup> mutants 342 333 128 164 264 254 254 138 122	no URA3 mutations 105 35 4 18 18 123 38 38 0 22	URA3 mutations 237 306 125 154 154 124 224 224 138 102	Trai # 24 92 25 64 36 83 5 30	nsitions rate 0.12 50 420x 1.5 98 66x 6.3 ≤ 0.001 0.12 59 480x 0.51 69 140x	Fran # 12 162 7 67 67 14 85 7 7 54	neshifts rate 0.060 88 <b>1,500x</b> 0.42 100 <b>250x</b> <b>6.0</b> 0.009 0.047 60 <b>1,300x</b> 0.71 120 <b>170x</b>
URA3 orientation 2 URA3 orientation 1 3	2012 RNH201 a 2 mh2011 RNH201 a 2012 RNH201 2012 C	<b>-M644G</b> Δ/wt MMR wt MMR msh2Δ correction fact wt MMR msh3,6Δ correction fact ilues wt MMR msh2Δ correction fact wt MMR msh3,6Δ correction fact	mutation rate (x10 <sup>-7</sup> ) 1.7 180 tors 7.6 250 tors correction factor 0.83 180 tors 14 280 cors :orrection factor	95% confidence (1.3-2.3) (120-270) (4.3-10) (170-350) (170-350) (0.71-0.96) (100-220) (100-220) (9.8-20) (210-360) <i>yr ratios</i>	5-FOA <sup>R</sup> mutants 342 333 128 164 246 254 254 138 122	no URA3 mutations 105 35 4 18 18 123 38 38 0 22	URA3 mutations 237 306 125 154 154 224 224 124 224 138 102	Trai # 24 92 25 64 36 83 5 30	rate 0.12 50 420x 1.5 98 66x 6.3 ≤ 0.001 0.12 59 480x 0.51 69 140x 3.6	Fran # 12 162 7 67 67 14 85 7 54	neshifts rate 0.060 88 <b>1,500x</b> 0.42 100 <b>250x</b> <b>6.0</b> 0.009 0.047 60 <b>1,300x</b> 0.71 120 <b>170x</b> <b>7.3</b>

Some data is repeated from previous tables and between panels. For comparison, all rates are listed by strain in Table S6. All rates are multiplied by  $10^7$ . C.f. stands for correction factor. Correction factors are compared in Fig. 2A, listed as "*msh3,6*/wt MMR" (above) and "*msh2*/wt MMR" (below). Strain origins and related references may be found in Table S5.

	pol3-L612M		mutation	95%	5-FOA <sup>R</sup>	no URA3	URA3	Tran	sitions	Fram	eshifts
т	sh64	۵/wt MMR	rate (x10 <sup>-7</sup> )	confidence	mutants	change	mutations	#	rate	#	rate
	11	wt MMR	1.4	(1.1-1.8)	245	72	173	112	0.64	20	0.11
1	NH2(	msh6∆	170	(130-230)	313	14	310	271	150	6	3.3
tion	RI	correction fact	tors						230x		29x
enta	14	wt MMR	1.4	(0.97-2.1)	151	28	123	60	0.56	22	0.20
URA3 orie	h20	msh6∆	180	(130-260)	142	13	132	117	150	8	10
	n	correction fact	tors						270x		50x
n	RNH	H201/rnh201∆ c	orrection facto		0.9		0.6				
	p-va	lues							0.370		0.230
	01	wt MMR	2.30	(2.0-2.7)	243	45	198	87	0.82	34	0.32
2	NH2	msh6∆	160	(130-210)	354	27	335	241	110	5	2.3
tion	R	correction fact	tors						130x		7.0x
enta	14	wt MMR	1	(0.84-1.5)	188	45	143	45	0.26	23	0.13
8 orie	h20	msh6∆	180	(140-230)	70	4	71	57	150	1	2.6
IRAS	rn	correction fact	tors						570x		19x
C C	RNH	H201/rnh201∆ c	correction facto	or ratios					0.23		0.37
	p-va	lues							≤ 0.001		0.021

Table S3. *pol3-L612M* mutation rates in two *URA3* orientations ± *MSH6* and/or *RNH201*, Related to Figure 2

For comparison, all rates are listed by strain in Table S6. All rates are multiplied by  $10^7$ . Correction factors are compared in Fig. 2B, listed as "*msh6*//wt MMR". Strain origins and related references may be found in Table S5.

Table S4. Mutation rat	tes in a wild type polymeras	e background for tw	o URA3 orientations ±
MMR and/or RNH201,	, Related to Figure 2	-	

wild type pols		ype pols	mutation	95%	5-FOA <sup>R</sup>	no URA3	URA3	Trar	nsitions	Fram	Frameshifts	
ms	sh3,6	Δ/wt MMR	rate (x10 <sup>-7</sup> )	confidence	mutants	change	mutations	#	rate	#	rate	
	11	wt MMR	0.2	(0.14-0.23)	211	70	141	47	0.040	11	0.0094	
-	VH2(	msh3,6∆	8	(6.1-11)	228	63	170	38	1.4	100	3.6	
tion	R	correction fact	tors						34x		380x	
entat	77	wt MMR	0.3	(0.22-0.46)	251	95	156	32	0.041	27	0.034	
orie	h20	msh3,6∆	10	(6.5-14)	298	92	206	76	2.4	98	3.2	
IRA 3	E	correction fact	tors						60x		92x	
2	RNł	<del>1</del> 201/rnh201∆ c	correction factor	or ratios					0.6		4.1	
	p-va	lues							0.083		≤ 0.001	
	01	wt MMR	0.21	(0.16-0.27)	257	99	158	43	0.035	14	0.011	
7	NH2	msh3,6∆	8	(6.1-10)	258	79	186	32	0.97	113	3.4	
tion	R	correction fact	tors						28x		300x	
A3 orientat	14	wt MMR	1	(0.43-0.75)	273	62	211	32	0.075	14	0.033	
	h20	msh3,6∆	12	(8.8-17)	244	49	196	37	1.8	99	4.9	
IRA3	rn	correction fact	correction factors								150x	
2	RNH201/rnh201∆ correction factor ratios										2.0	
	p-va	lues							0.38		0.069	
v	vild t	ype pols	mutation	95%	5-FOA <sup>R</sup>	no URA3	URA3	Trar	sitions	Fram	neshifts	
v m	vild t sh2/	ype pols ⁄/wt MMR	mutation rate (x10 <sup>-7</sup> )	95% confidence	5-FOA <sup>R</sup> mutants	no URA3 change	URA3 mutations	Trar #	nsitions rate	Fran #	neshifts rate	
v m	vild t sh24	ype pols 2/wt MMR wt MMR	mutation rate (x10 <sup>-7</sup> ) 0.2	95% confidence (0.14-0.23)	5-FOA <sup>R</sup> mutants 211	no URA3 change 70	URA3 mutations 141	Trar # 47	nsitions rate 0.040	Fran # 11	neshifts rate 0.0094	
w m	vild t sh21	ype pols Δ/wt MMR wt MMR msh2Δ	mutation rate (x10 <sup>-7</sup> ) 0.2 7	95% confidence (0.14-0.23) (3.5-15)	5-FOA <sup>R</sup> mutants 211 195	no URA3 change 70 15	URA3 mutations 141 180	Trar # 47 41	rate 0.040 1.5	Fram # 11 118	neshifts rate 0.0094 4.4	
tion 1 w	vild t sh21	ype pols Δ/wt MMR wt MMR msh2Δ correction fact	mutation rate (x10 <sup>-7</sup> ) 0.2 7 tors	95% confidence (0.14-0.23) (3.5-15)	5-FOA <sup>R</sup> mutants 211 195	no URA3 change 70 15	URA3 mutations 141 180	Trar # 47 41	nsitions rate 0.040 1.5 <b>38x</b>	Fran # 11 118	neshifts rate 0.0094 4.4 460x	
entation 1 <i>w</i> s	vild t sh21 Losh21 VILTOT	ype pols Δ/wt MMR wt MMR msh2Δ correction fact wt MMR	mutation rate (x10 <sup>-7</sup> ) 0.2 7 tors 0.3	95% confidence (0.14-0.23) (3.5-15) (0.22-0.46)	5-FOA <sup>R</sup> mutants 211 195 251	no URA3 change 70 15 95	URA3 mutations 141 180 156	Trar # 47 41 32	rate 0.040 1.5 38x 0.041	Fran # 11 118 27	neshifts rate 0.0094 4.4 460x 0.034	
8 orientation 1	h2010 RNH201 Diag	ype pols Δ/wt MMR wt MMR msh2Δ correction fact wt MMR msh3,6Δ	mutation rate (x10 <sup>-7</sup> ) 0.2 7 tors 0.3 10	95% confidence (0.14-0.23) (3.5-15) (0.22-0.46) (6.5-14)	5-FOA <sup>R</sup> mutants 211 195 251 298	no URA3 change 70 15 95 92	URA3 mutations 141 180 156 206	Trar # 47 41 32 76	rate 0.040 1.5 38x 0.041 2.4	Fran # 11 118 27 98	neshifts rate 0.0094 4.4 460x 0.034 3.2	
IRA3 or ientation 1 3 <	vild t sh201 VH201 RNH201	ype pols Δ/wt MMR wt MMR msh2Δ correction fact wt MMR msh3,6Δ correction fact	mutation rate (x10 <sup>-7</sup> ) 0.2 7 tors 0.3 10 tors	95% confidence (0.14-0.23) (3.5-15) (0.22-0.46) (6.5-14)	5-FOA <sup>R</sup> mutants 211 195 251 298	no URA3 change 70 15 95 92	URA3 mutations 141 180 156 206	Trar # 47 41 32 76	asitions           rate           0.040           1.5           38x           0.041           2.4           60x	Fran # 11 118 27 98	neshifts rate 0.0094 4.4 460x 0.034 3.2 92x	
URA3 orientation 1	vild t sh21 RNH5017 RNH5017	ype pols Δ/wt MMR wt MMR msh2Δ correction fact wt MMR msh3,6Δ correction fact	mutation rate (x10 <sup>-7</sup> ) 0.2 7 tors 0.3 10 tors correction factor	95% confidence (0.14-0.23) (3.5-15) (0.22-0.46) (6.5-14) pr ratios	5-FOA <sup>R</sup> mutants 211 195 251 298	no URA3 change 70 15 95 92	URA3 mutations 141 180 156 206	Trar # 47 41 32 76	rate 0.040 1.5 38x 0.041 2.4 60x 0.63	Fran # 11 118 27 98	neshifts rate 0.0094 4.4 460x 0.034 3.2 92x 5.1	
URA3 orientation 1 3 <	vild t sh21 INH5017 RNH5017 P-va	ype pols (wt MMR) wt MMR msh2 correction fact wt MMR msh3,6 correction fact correction fact dues	mutation rate (x10 <sup>-7</sup> ) 0.2 7 tors 0.3 10 tors correction factor	95% confidence (0.14-0.23) (3.5-15) (0.22-0.46) (6.5-14) or ratios	5-FOA <sup>R</sup> mutants 211 195 251 298	no URA3 change 70 15 95 92	URA3 mutations 141 180 156 206	Trar # 47 41 32 76	rate       0.040       1.5       38x       0.041       2.4       60x       0.63       0.17	Fran # 11 118 27 98	neshifts rate 0.0094 4.4 460x 0.034 3.2 92x 5.1 ≤ 0.001	
URA3 orientation 1 3 <	vild t sh22 P-va p-va	ype pols Δ/wt MMR wt MMR msh2Δ correction fact wt MMR msh3,6Δ correction fact H201/rnh201Δ content ilues wt MMR	mutation           rate (x10 <sup>-7</sup> )           0.2           7           tors           0.3           10           tors           correction factor           0.21	95% confidence (0.14-0.23) (3.5-15) (0.22-0.46) (6.5-14) or ratios (0.16-0.27)	5-FOA <sup>R</sup> mutants 211 195 251 298 257	no URA3 change 70 15 95 92 92	URA3 mutations 141 180 156 206 206	Trar # 47 41 32 76 43	rate 0.040 1.5 38x 0.041 2.4 60x 0.63 0.17 0.035	Fran # 11 118 27 98 14	neshifts rate 0.0094 4.4 460x 0.034 3.2 92x 5.1 ≤ 0.001 0.011	
2 URA3 orientation 1 3 <	NH201 t p-va p-va	ype pols Δ/wt MMR wt MMR msh2Δ correction fact wt MMR msh3,6Δ correction fact H201/rnh201Δ content ilues wt MMR msh2Δ	mutation           rate (x10 <sup>-7</sup> )           0.2           7           tors           0.3           10           tors           0.2           0.3           10           tors           0.2           10           tors           0.2           10           tors           0.21           10	95% confidence (0.14-0.23) (3.5-15) (0.22-0.46) (6.5-14) or ratios (0.16-0.27) (6.7-13)	5-FOA <sup>R</sup> mutants 211 195 251 298 298 257 257	no URA3 change 70 15 95 92 92 99 99	URA3 mutations 141 180 156 206 206 158 181	Trar # 47 41 32 76 43 43 23	rate         0.040         1.5         38x         0.041         2.4         60x         0.17         0.035         1.1	Fran # 11 118 27 98 98 14 14	neshifts rate 0.0094 4.4 460x 0.034 3.2 92x 5.1 ≤ 0.001 0.011 5.6	
tion 2 URA3 orientation 1 3 <	RNH201 - A - A - A - A - A - A - A - A - A - A	ype pols Δ/wt MMR wt MMR msh2Δ correction fact wt MMR msh3,6Δ correction fact dues wt MMR msh2Δ correction fact	mutation           rate (x10 <sup>-7</sup> )           0.2           7           tors           0.3           10           tors           0.21           10           tors	95% confidence (0.14-0.23) (3.5-15) (0.22-0.46) (6.5-14) or ratios (0.16-0.27) (6.7-13)	5-FOA <sup>R</sup> mutants 211 195 251 298 257 257 193	no URA3 change 70 15 95 92 92 99 99	URA3 mutations 141 180 156 206 206 158 158	Trar # 47 41 32 76 43 43 23	rate 0.040 1.5 38x 0.041 2.4 60x 0.63 0.17 0.035 1.1 32x	Fran # 11 118 27 98 14 113	neshifts rate 0.0094 4.4 460x 0.034 3.2 92x 5.1 ≤ 0.001 0.011 5.6 490x	
entation 2 URA3 orientation 1 3 <	10 RNH201 d rnh2010 RNH201 d rnh201 RNH201 d rnh201 d rnh201 d rnh2010 RNH201 d rnh201 rnh201 rnh201 d	ype pols Δ/wt MMR wt MMR msh2Δ correction fact wt MMR msh3,6Δ correction fact H201/rnh201Δ content ilues wt MMR msh2Δ correction fact wt MMR	mutation           rate (x10 <sup>-7</sup> )           0.2           7           tors           0.3           10           tors           0.21           10           tors	95% confidence (0.14-0.23) (3.5-15) (0.22-0.46) (6.5-14) or ratios (0.16-0.27) (6.7-13) (0.43-0.75)	5-FOA <sup>R</sup> mutants 211 195 251 298 257 193 273	no URA3 change 70 15 95 92 92 99 12 12 62	URA3 mutations 141 180 156 206 206 158 181 181 211	Trar # 47 41 32 76 43 43 23 32	nsitions         rate         0.040         1.5         38x         0.041         2.4         60x         0.633         0.17         0.035         1.1         32x         0.075	Fran # 11 118 27 98 14 113 14	neshifts rate 0.0094 4.4 460x 0.034 3.2 92x 5.1 ≤ 0.001 0.011 5.6 490x 0.033	
3 orientation 2 URA3 orientation 1 3 <	h201Δ RNH201 d X rnh201Δ RNH201 Signature	ype pols Δ/wt MMR wt MMR msh2Δ correction fact wt MMR msh3,6Δ correction fact dues wt MMR msh2Δ correction fact wt MMR msh3,6Δ	mutation           rate (x10 <sup>-7</sup> )           0.2           7           tors           0.3           10           tors           0.21           10           tors	95% confidence (0.14-0.23) (3.5-15) (0.22-0.46) (6.5-14) or ratios (0.16-0.27) (6.7-13) (0.43-0.75) (8.8-17)	5-FOA <sup>R</sup> mutants 211 195 251 298 257 193 257 193 273 244	no URA3 change 70 15 95 92 92 92 92 12 62 49	URA3 mutations 141 180 156 206 206 158 181 158 181 211 196	Trar # 47 41 32 76 43 43 23 32 32 37	rate         0.040         1.5         38x         0.041         2.4         60x         0.63         0.17         0.035         1.1         32x         0.075         1.8	Fran # 11 118 27 98 98 14 113 14 99	neshifts rate 0.0094 4.4 460x 0.034 3.2 92x 5.1 ≤ 0.001 0.011 5.6 490x 0.033 4.9	
IRA3 orientation 2 URA3 orientation 1 3 <	<i>Thiso1∆ RNH201 d N Thiso1∆ RNH201 f P h h h h h h h h h h</i>	ype pols Δ/wt MMR wt MMR msh2Δ correction fact wt MMR msh3,6Δ correction fact H201/rnh201Δ correction fact wt MMR msh2Δ correction fact wt MMR msh3,6Δ correction fact	mutation           rate (x10 <sup>-7</sup> )           0.2           7           tors           0.3           10           tors           0.21           10           tors           12           tors	95% confidence (0.14-0.23) (3.5-15) (0.22-0.46) (6.5-14) (6.5-14) or ratios (0.16-0.27) (6.7-13) (0.43-0.75) (8.8-17)	5-FOA <sup>R</sup> mutants 211 195 251 298 257 193 257 193 273 244	no URA3 change 70 15 95 92 92 92 12 62 49	URA3 mutations 141 180 206 206 158 181 181 211 196	Trar # 47 41 32 76 43 23 43 23 32 37	rate 0.040 1.5 38x 0.041 2.4 60x 0.63 0.17 0.035 1.1 32x 0.075 1.8 24x	Fran # 11 118 27 98 7 98 14 113 14 99	neshifts rate 0.0094 4.4 460x 0.034 3.2 92x 5.1 ≤ 0.001 0.011 5.6 490x 0.033 4.9 150x	
URA3 orientation 2 URA3 orientation 1 3 <	<i>Thisold</i> RNH201 d NH201 and RNH201 RNH201 RNH201 and RNH201 and RNH201 and RNH201 RNH201 RNH201 and RNH201 a	ype pols Δ/wt MMR msh2Δ correction fact wt MMR msh3,6Δ correction fact dues wt MMR msh2Δ correction fact wt MMR msh3,6Δ correction fact	mutation           rate (x10 <sup>-7</sup> )           0.2           7           tors           0.3           10           tors           0.21           10           tors           0.21           10           tors           correction factor           12           tors	95% confidence (0.14-0.23) (3.5-15) (0.22-0.46) (6.5-14) or ratios (0.16-0.27) (6.7-13) (0.43-0.75) (8.8-17) or ratios	5-FOA <sup>R</sup> mutants 211 195 251 298 257 193 257 193 273 244	no URA3 change 70 15 95 92 92 92 92 12 62 49	URA3 mutations 141 180 206 206 158 181 158 181 211 196	Trar # 47 41 32 76 43 23 43 23 32 37	rate 0.040 1.5 38x 0.041 2.4 60x 0.63 0.17 0.035 1.1 32x 0.075 1.8 24x 1.3	Fran # 11 118 27 98 27 98 14 113 14 99	neshifts rate 0.0094 4.4 460x 0.034 3.2 92x 5.1 ≤ 0.001 0.011 5.6 490x 0.033 4.9 150x 3.3	

Some data is repeated from previous tables and between panels. For comparison, all rates are listed by strain in Table S6. All rates are multiplied by  $10^7$ . Correction factors are compared in Fig. 2C, listed as "*msh3,6*Δ/wt MMR" (above) and "*msh2*Δ/wt MMR" (below). Strain origins and related references may be found in Table S5.

strain	strain origin	ploidy	polymerase alleles	other genotype notes	data set origin
h001	see reference and dissected from diploid d011 or d041 or d081	haploid	wild type	URA3 OR1	expanded from (Nick McElhinny et al., 2010a)
h002	see reference and dissected from diploid d012 or d042 or d082	haploid	wild type	URA3 OR2	expanded from (Nick McElhinny et al., 2010a)
h011	see reference and dissected from diploid d011	haploid	wild type	msh2Δ URA3 OR1	expanded from (Nick McElhinny et al., 2010a)
h012	see reference and dissected from diploid d012	haploid	wild type	msh2Δ URA3 OR2	expanded from (Nick McElhinny et al., 2010a)
h041	dissected from diploid d041 or d081	haploid	wild type	msh3,6Δ URA3 OR1	this study
h042	dissected from diploid d042 or d082	haploid	wild type	msh3,6Δ URA3 OR2	this study
h051	see reference (SNM106)	haploid	wild type	rnh201∆ URA3 OR1	from (Clark et al., 2011)
h052	see reference (SNM114)	haploid	wild type	rnh201∆ URA3 OR2	from (Clark et al., 2011)
h081	dissected from diploid d081	haploid	wild type	msh3,6Δ rnh201Δ URA3 OR1	this study
h082	dissected from diploid d082	haploid	wild type	msh3,6Δ rnh201Δ URA3 OR2	this study
h201	see reference (DTA201) and dissected from diploid d211	haploid	pol2-M644G	URA3 OR1	expanded from (Pursell et al., 2007)
h202	see reference (DTA202) and dissected from diploid d212	haploid	pol2-M644G	URA3 OR2	expanded from (Pursell et al., 2007)
h201ML	SNM82 (Nick McElhinny et al., 2010b)	haploid	pol2-M644L	URA3 OR1	this study
h202ML	SNM90 (Nick McElhinny et al., 2010b)	haploid	pol2-M644L	URA3 OR2	this study
h211	DTA201 and dissected from diploid d211	haploid	pol2-M644G	msh2∆ URA3 OR1	from (Lujan et al., 2012)
h212	DTA202 and dissected from diploid d212	haploid	pol2-M644G	msh2∆ URA3 OR2	from (Lujan et al., 2012)
h221	see reference (DTA201 $\Delta$ msh3) and dissected from diploid d281	haploid	pol2-M644G	msh3∆ URA3 OR1	from (Clark et al., 2011)
h222	see reference (DTA202 $\Delta$ msh3) and dissected from diploid d282	haploid	pol2-M644G	msh3∆ URA3 OR2	from (Clark et al., 2011)
h231	dissected from diploid d281	haploid	pol2-M644G	msh6Δ URA3 OR1	this study
h232	dissected from diploid d282	haploid	pol2-M644G	msh6Δ URA3 OR2	this study
h241	dissected from diploid d281	haploid	pol2-M644G	msh3,6Δ URA3 OR1	this study
h242	dissected from diploid d282	haploid	pol2-M644G	msh3,6Δ URA3 OR2	this study
h251	see reference (SNM120) and dissected from diploid d281	haploid	pol2-M644G	rnh201Δ URA3 OR1	expanded from (Nick McElhinny et al., 2010b)
h252	see reference (SHM127) and dissected from diploid d282	haploid	pol2-M644G	rnh201Δ URA3 OR2	expanded from (Nick McElhinny et al., 2010b)
h251ML	transformation of h201ML with HYG-R with RNH201 flanking DNA (Nick McElhinny et al., 2010b)	haploid	pol2-M644L	rnh201∆ URA3 OR1	this study
h252ML	transformation of h201ML with HYG-R with RNH201 flanking DNA (Nick McElhinny et al., 2010b)	haploid	pol2-M644L	rnh201Δ URA3 OR2	this study
h261	see reference and dissected from diploid d281	haploid	pol2-M644G	msh3Δ rnh201Δ URA3 OR1	expanded from (Clark et al., 2011)

## Table S5. Strains used in this study, Related to Figures 1 and 2 and Table 1

h262	see reference and dissected from diploid d282	haploid	pol2-M644G	msh3∆ rnh201∆ URA3 OR2	expanded from (Clark et al., 2011)
h271	see reference and dissected from diploid d281	haploid	pol2-M644G	msh6Δ rnh201Δ URA3 OR1	from (Clark et al., 2011)
h272	see reference and dissected from diploid d282	haploid	pol2-M644G	msh6Δ rnh201Δ URA3 OR2	from (Clark et al., 2011)
h281	dissected from diploid d281	haploid	pol2-M644G	msh3,6Δ rnh201Δ URA3 OR1	expanded from (Clark et al., 2011)
h282	dissected from diploid d282	haploid	pol2-M644G	msh3,6Δ rnh201Δ URA3 OR2	expanded from (Clark et al., 2011)
h301	see reference (DTA301) and dissected from diploid d341 or d371	haploid	pol3-L612M	URA3 OR1	expanded from (Nick McElhinny et al., 2008; Nick McElhinny et al., 2010a)
h302	see reference (DTA302) and dissected from diploid d342 or d372	haploid	pol3-L612M	URA3 OR2	expanded from (Nick McElhinny et al., 2008; Nick McElhinny et al., 2010a)
h331	dissected from diploid d341 or d371	haploid	pol3-L612M	msh6Δ URA3 OR1	this study
h332	dissected from diploid d342 or d372	haploid	pol3-L612M	msh6Δ URA3 OR2	this study
h351	see reference (JSW11) and dissected from diploid d371	haploid	pol3-L612M	rnh201∆ URA3 OR1	this study
h352	see reference (JSW15) and dissected from diploid d372	haploid	pol3-L612M	rnh201∆ URA3 OR2	this study
h371	dissected from diploid d371	haploid	pol3-L612M	msh6Δ rnh201Δ URA3 OR1	this study
h372	dissected from diploid d372	haploid	pol3-L612M	msh6Δ rnh201Δ URA3 OR2	this study
d011	also known as ySNM_1025	diploid	wild type	msh2Δ/MSH2 URA3 OR1	from (Nick McElhinny et al., 2008)
d012	also known as ySNM_1028	diploid	wild type	msh2Δ/MSH2 URA3 OR2	from (Nick McElhinny et al., 2008)
d041	derived from mating of h031 and h021	diploid	wild type	msh3,6Δ/MSH3,6 URA3 OR1	this study
d042	derived from mating of h032 and h022	diploid	wild type	msh3,6Δ/MSH3,6 URA3 OR2	this study
d081	derived from mating of h041 and h051	diploid	wild type	msh3/MSH3 msh6Δ/MSH6 rnh201Δ/RNH201 URA3 OR1	this study
d082	derived from mating of h042 and h052	diploid	wild type	msh3/MSH3 msh6Δ/MSH6 rnh201Δ/RNH201 URA3 OR2	this study
h021	also known as DTA101∆msh3	haploid	wild type	msh3∆ URA3 OR1	from (Clark et al., 2011)
h022	also known as DTA102Δmsh3	haploid	wild type	msh3∆ URA3 OR2	from (Clark et al., 2011)
h031	dissected from d031	haploid	wild type	msh6∆ URA3 OR1	this study
h032	dissected from d032	haploid	wild type	msh6Δ URA3 OR2	this study
d031	transformation of ySNM_1001 with TRP1 with MSH6 flanking DNA	diploid	wild type	msh6Δ/MSH6 URA3 OR1	this study
d032	transformation of ySNM_1005 with TRP1 with MSH6 flanking DNA	diploid	wild type	msh6Δ/MSH6 URA3 OR2	this study
ySNM 1001	transformation of DTA101 with plasmid YEpHO	diploid	wild type	URA3 OR1	from (Nick McElhinny et al., 2008)
ySNM 1005	transformation of DTA102 with plasmid YEpHO	diploid	wild type	URA3 OR2	from (Nick McElhinny et al., 2008)
d341	derived from mating of h331 and h321	diploid	pol3-L612M	msh3,6Δ/MSH3,6 URA3 OR1	this study
d342	derived from mating of h332 and h322	diploid	pol3-L612M	msh3,6Δ/MSH3,6 URA3 OR2	this study
d371	derived from mating of h331 and h351	diploid	pol3-L612M	msh6Δ/MSH6 rnh201Δ/RNH201 URA3 OR1	this study

d372	derived from mating of h332 and h352	diploid	pol3-L612M	msh6Δ/MSH6 rnh201Δ/RNH201 URA3 OR2	this study
h331	dissected from d331	haploid	pol3-L612M	msh6Δ URA3 OR1	this study
h332	dissected from d332	haploid	pol3-L612M	msh6Δ URA3 OR2	this study
d331	transformation of ySNM_1018 with TRP1 with MSH6 flanking DNA	diploid	pol3-L612M	msh6Δ/MSH6 URA3 OR1	this study
d332	transformation of ySNM_1021 with TRP1 with MSH6 flanking DNA	diploid	pol3-L612M	msh6Δ/MSH6 URA3 OR2	this study
h321	also known as DTA301∆msh3	haploid	pol3-L612M	msh3∆ URA3 OR1	this study
h322	also known as DTA302∆msh3	haploid	pol3-L612M	msh3Δ URA3 OR2	this study
d211	derived from mating of DTA201 and h211	diploid	pol2-M644G	msh2Δ/MSH2 URA3 OR1	this study
d212	derived from mating of DTA202 and h212	diploid	pol2-M644G	msh2Δ/MSH2 URA3 OR2	this study
d281	derived from mating of h241 and h251	diploid	pol2-M644G	msh3,6Δ/MSH3,6 rnh201Δ/RNH201 URA3 OR1	this study
d282	derived from mating of h242 and h252	diploid	pol2-M644G	msh3,6Δ/MSH3,6 rnh201Δ/RNH201 URA3 OR2	this study
h241	dissected from d241	haploid	pol2-M644G	msh3,6Δ URA3 OR1	this study
h242	dissected from d242	haploid	pol2-M644G	msh3,6Δ URA3 OR2	this study
d241	derived from mating h231 and h221	diploid	pol2-M644G	msh3,6Δ/MSH3,6 URA3 OR1	this study
d242	derived from mating h232 and h222	diploid	pol2-M644G	msh3,6Δ/MSH3,6 URA3 OR2	this study
h231	derived from dissection of d231	haploid	pol2-M644G	msh6Δ URA3 OR1	this study
h232	derived from dissection of d232	haploid	pol2-M644G	msh6Δ URA3 OR2	this study
d231	derived from dissection and back cross of d231.5	diploid	pol2-M644G	msh6Δ/MSH6 URA3 OR1	this study
d232	derived from dissection and back cross of d232.5	diploid	pol2-M644G	msh6Δ/MSH6 URA3 OR2	this study
d231.5	derived from mating h201 and h031	diploid	pol2-M644G/POL2	msh6Δ/MSH6 URA3 OR1	this study
d232.5	derived from mating h202 and h032	diploid	pol2-M644G/POL2	msh6Δ/MSH6 URA3 OR2	this study

All strains are descended from  $\Delta|(-2)|$ -7B-YUNI300 (Pavlov et al., 2001) and have the following genotypic markers (homozygous in diploids): *CAN1, his7-2, leu2-* $\Delta$ ::*kanMX, ura3-* $\Delta$ ::, *trp1-289, ade2-1, lys2-* $\Delta$ *GG2899-2900. OR1* and *OR2* refer to *URA3* orientations at the *AGP1* locus on *Saccharomyces cerevisiae* chromosome 3: the *URA3* coding strand is the leading strand template in *OR1* and the lagging strand template in *OR2*.

			MMR	mutation	95%	5-FOA <sup>R</sup>	wild type	URA3	Trans	sitions	Fram	neshifts
		RNH201	alleles	rate (x10 <sup>-7</sup> )	confidence	mutants	URA3	mutations	count	rate	count	rate
	11	+	wt	1.7	(1.3-2.3)	342	105	237	24	0.12	12	0.060
	OF	+	msh6∆	110	(82-150)	219	26	205	130	65	12	6.0
	RA3	Δ	wt	7.6	(4.3-10)	128	4	125	25	1.5	7	0.42
	Ŋ	Δ	msh6∆	170	(130-220)	141	10	133	82	99	7	8.4
	22	+	wt	0.83	(0.71-0.96)	246	123	124	36	0.12	14	0.047
	S OF	+	msh6∆	54	(40-75)	181	38	149	79	24	10	3.0
	RA3	Δ	wt	14	(9.8-20)	138	0	138	5	0.51	7	0.71
ð	Ď	Δ	msh6∆	81	(57-110)	134	20	126	65	39	3	1.8
1644	11	+	msh3∆	1.6	(1.1-2.5)	122	15	109	9	0.12	50	0.66
12-N	I OF	+	msh3,6∆	110	(88-140)	173	16	166	67	43	56	36
od	RA3	Δ	msh3∆	11	(8.8-14)	137	17	120	19	1.5	26	2.1
	5	Δ	msh3,6∆	250	(170-350)	164	18	154	64	98	67	100
	5	+	msh3∆	2.9	(2.2-3.9)	125	17	108	4	0.093	88	2.0
	I OR	+	msh3,6∆	280	(220-370)	158	16	144	34	60	80	140
	RA3	Δ	msh3∆	15	(11-21)	139	21	118	3	0.32	33	3.6
	5	Δ	msh3,6∆	280	(210-360)	122	22	102	30	69	54	120
	OR1	+	msh2∆	180	(120-270)	333	35	306	92	50	162	88
	OR2	+	msh2∆	180	(100-220)	254	38	224	83	59	85	60
	OR1	+	wt	1.4	(1.1-1.8)	245	72	173	112	0.64	20	0.11
		+	msh6∆	170	(130-230)	313	14	310	271	150	6	3.3
W	RA3	Δ	wt	1.4	(0.97-2.1)	151	28	123	60	0.56	22	0.20
.612	Ď	Δ	msh6∆	180	(130-260)	142	13	132	117	150	8	10
13-L	2	+	wt	2.3	(2.0-2.7)	243	45	198	87	0.82	34	0.32
bd	I OR	+	msh6∆	160	(130-210)	354	27	335	241	110	5	2.3
	RA3	Δ	wt	1.1	(0.84-1.5)	188	45	143	45	0.26	23	0.13
	5	Δ	msh6∆	180	(140-230)	70	4	71	57	150	1	2.6
	ч	+	wt	0.2	(0.14-0.23)	211	70	141	47	0.040	11	0.0094
	I OR	+	msh3,6∆	8.1	(6.1-11)	228	63	170	38	1.4	100	3.6
ses	RA3	Δ	wt	0.3	(0.22-0.46)	251	95	156	32	0.041	27	0.034
nera	Ď	Δ	msh3,6∆	10	(6.5-14)	298	92	206	76	2.4	98	3.2
lyn	22	+	wt	0.21	(0.16-0.27)	257	99	158	43	0.035	14	0.011
e bć	I OF	+	msh3,6∆	7.8	(6.1-10)	258	79	186	32	0.97	113	3.4
typ	RA3	Δ	wt	0.64	(0.43-0.75)	273	62	211	32	0.075	14	0.033
wild	5	Δ	msh3,6∆	12	(8.8-17)	244	49	196	37	1.8	99	4.9
	OR1	+	msh2∆	7.2	(3.5-15)	195	15	180	41	1.5	118	4.4
	OR2	+	msh2∆	10	(6.7-13)	193	12	181	23	1.1	113	5.6
	۲1	+	wt	0.39	(0.30-0.52)	87	39	49	8	0.036	10	0.045
12- 4GL	ð	Δ	wt	0.50	(0.37-0.66)	83	31	52	17	0.10	7	0.042
po N64	۲1	+	wt	0.61	(0.53-0.71)	101	33	69	17	0.10	18	0.11
	i0	Δ	wt	0.50	(0.45-0.56)	93	46	47	27	0.15	4	0.022

 Table S6. Mutation rates, Related to Figure 2

All rates are multiplied by 10<sup>7</sup>. Strain origins and related references may be found in Table S5.

	NO MEAN	mutation	95%	5-FOA <sup>R</sup>	no URA3	URA3	±1-bas	se Indels	2-5 bp	Deletions
p012-11/044L		rate (x10 <sup>-7</sup> )	confidence	mutants	change	mutations	#	rate	#	rate
01	URA3 OR1	0.39	(0.30-0.52)	87	39	49	10	0.045	3	0.0049
NH2	URA3 OR2	0.50	(0.37-0.66)	83	31	52	7	0.042	1	0.0060
RI	combined	0.44	(0.33-0.58)	170	70	101	17	0.043	4	0.0052
14	URA3 OR1	0.61	(0.53-0.71)	101	33	69	18	0.11	9	0.0060
h20	URA3 OR2	0.50	(0.45-0.56)	93	46	47	4	0.022	1	0.0054
rn	combined	0.55	(0.49-0.63)	194	79	116	22	0.048	10	0.0057
incr	ease upon RNI	H201 deletion						1.1x		1.9x

Table S7. Mutation rates in a *pol2-M644L* background for two *URA3* orientations  $\pm$  *RNH201*, Related to Figure 2

For comparison, some rates are listed by strain in Table S6. All rates are multiplied by  $10^7$ . "Combined" rates are geometric means of rates from two *URA3* orientations (mutation rates from forward mutation experiments are lognormally distributed). Fold-increases in rates upon *RNH201* deletion are compared in Fig. 2D. Strain origins and related references may be found in Table S5.

#### **Supplemental References**

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