**SUPPLEMENTARY MATERIAL**

**Table S1.** Mutation rates calculated for specific mutation types in the *URA3* sequence in the *rev3*Δ *msh6*Δ background. Mutation spectra for strains OR1, OR2, *pol3-L612M* OR1, *pol3-L612M* OR2, *pol2-M644G* OR1, and *pol2-M644G* OR2 were presented previously in [44] and [50].

| | OR1 | | OR2 | | *dpb2-100* OR1 | | *dpb2-100* OR2 | | *pol3-L612M* OR1 | | *pol3-L612M* OR2 | | *dpb2-100 pol3-L612M* OR1 | | *dpb2-100 pol3-L612M* OR2 | | *pol2-M644G* OR1 | | *pol2-M644G* OR2 | | *dpb2-100 pol2-M644G* OR1 | | *dpb2-100 pol2-M644G* OR2 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Transitions** | 103[a] | **1.10**[b] | 37 | **0.53** | 80 | **9.49** | 67 | **7.54** | 161 | **35.42** | 112 | **22.76** | 119 | **59.73** | 96 | **76.03** | 83 | **28.09** | 68 | **26.12** | 98 | **52.30** | 84 | **62.61** |
| T→C | 23 | **0.25** | 5 | **0.07** | 11 | **1.31** | 18 | **2.03** | 53 | **11.66** | 2 | **0.41** | 27 | **13.55** | 6 | **4.75** | 6 | **2.03** | 17 | **6.53** | 13 | **6.94** | 18 | **13.42** |
| *T→C at 97*[c] | *7* | ***0.07*** | *1* | ***0.01*** | *1* | ***0.12*** | *2* | ***0.23*** | *24* | ***5.28*** | *0* | ***0.00*** | *8* | ***4.02*** | *2* | ***1.58*** | 2 | ***0.68*** | 5 | ***1.92*** | 5 | ***2.67*** | 5 | ***3.73*** |
| *T→C at OS*[d] | *16* | ***0.17*** | *4* | ***0.06*** | *10* | ***1.19*** | *16* | ***1.80*** | *29* | ***6.38*** | *2* | ***0.41*** | *19* | ***9.54*** | *4* | ***3.17*** | 4 | ***1.35*** | 12 | ***4.61*** | 8 | ***4.27*** | 13 | ***9.69*** |
| A→G | 2 | **0.02** | 6 | **0.09** | 2 | **0.24** | 4 | **0.45** | 0 | **0.00** | 18 | **3.66** | 0 | **0.00** | 13 | **10.30** | 2 | **0.68** | 3 | **1.15** | 3 | **1.60** | 6 | **4.47** |
| C→T | 13 | **0.14** | 18 | **0.26** | 16 | **1.90** | 27 | **3.04** | 1 | **0.22** | 85 | **17.27** | 17 | **8.53** | 55 | **43.56** | 20 | **6.77** | 17 | **6.53** | 19 | **10.14** | 25 | **18.63** |
| *C→T at 310* | *4* | ***0.04*** | *11* | ***0.16*** | *5* | ***0.59*** | *11* | ***1.24*** | *1* | ***0.22*** | *43* | ***8.74*** | *5* | ***2.51*** | *40* | ***31.68*** | 6 | ***2.03*** | 9 | ***3.46*** | 4 | ***2.13*** | 15 | ***11.18*** |
| *C→T at OS* | *9* | ***0.10*** | *7* | ***0.10*** | *11* | ***1.31*** | *16* | ***1.80*** | *0* | ***0.00*** | *42* | ***8.54*** | *12* | ***6.02*** | *15* | ***11.88*** | 14 | ***4.74*** | 8 | ***3.07*** | 15 | ***8.00*** | 10 | ***7.45*** |
| G→A | 65 | **0.70** | 8 | **0.11** | 51 | **6.05** | 18 | **2.03** | 107 | **23.54** | 7 | **1.42** | 75 | **37.64** | 22 | **17.42** | 55 | **18.61** | 31 | **11.91** | 63 | **33.62** | 35 | **26.09** |
| *G→A at 764* | *18* | ***0.19*** | *2* | ***0.03*** | *8* | ***0.95*** | *2* | ***0.23*** | *45* | ***9.90*** | *2* | ***0.41*** | *15* | ***7.53*** | *3* | ***2.38*** | 10 | ***3.38*** | 9 | ***3.46*** | 12 | ***6.40*** | 4 | ***2.98*** |
| *G→A at OS* | *47* | ***0.50*** | *6* | ***0.09*** | *43* | ***5.10*** | *16* | ***1.80*** | *62* | ***13.64*** | *5* | ***1.02*** | *60* | ***30.11*** | *19* | ***15.05*** | 45 | ***15.23*** | 22 | ***8.45*** | 51 | ***27.22*** | 31 | ***23.11*** |
| **Transversions** | 54 | **0.58** | 87 | **1.24** | 37 | **4.39** | 55 | **6.19** | 8 | **1.76** | 54 | **10.97** | 17 | **8.53** | 33 | **26.14** | 69 | **23.35** | 35 | **13.44** | 57 | **30.42** | 49 | **36.52** |
| G→T | 25 | **0.27** | 70 | **0.99** | 20 | **2.37** | 50 | **5.63** | 2 | **0.44** | 47 | **9.55** | 8 | **4.02** | 22 | **17.42** | 37 | **12.52** | 23 | **8.83** | 26 | **13.87** | 24 | **17.89** |
| *G→T at 679/706* | *8* | ***0.09*** | *44* | ***0.63*** | *6* | ***0.71*** | *13* | ***1.46*** | *0* | ***0.00*** | *9* | ***1.83*** | *3* | ***1.51*** | *5* | ***3.96*** | 8 | ***2.71*** | 16 | ***6.15*** | 15 | ***8.00*** | 11 | ***8.20*** |
| *G→T at OS* | *17* | ***0.18*** | *26* | ***0.37*** | *14* | ***1.66*** | *37* | ***4.16*** | *2* | ***0.44*** | *38* | ***7.72*** | *5* | ***2.51*** | *17* | ***13.46*** | 29 | ***9.81*** | 7 | ***2.69*** | 11 | ***5.87*** | 13 | ***9.69*** |
| C→A | 14 | **0.15** | 9 | **0.13** | 8 | **0.95** | 0 | **0.00** | 4 | **0.88** | 0 | **0.00** | 4 | **2.01** | 2 | **1.58** | 2 | **0.68** | 2 | **0.77** | 11 | **5.87** | 11 | **8.20** |
| T→G | 9 | **0.10** | 4 | **0.06** | 2 | **0.24** | 2 | **0.23** | 1 | **0.22** | 0 | **0.00** | 2 | **1.00** | 0 | **0.00** | 2 | **0.68** | 3 | **1.15** | 1 | **0.53** | 4 | **2.98** |
| A→C | 2 | **0.02** | 1 | **0.01** | 1 | **0.12** | 1 | **0.11** | 0 | **0.00** | 3 | **0.61** | 0 | **0.00** | 1 | **0.79** | 0 | **0.00** | 0 | **0.00** | 3 | **1.60** | 0 | **0.00** |
| A→T | 2 | **0.02** | 0 | **0.00** | 1 | **0.12** | 1 | **0.11** | 1 | **0.22** | 0 | **0.00** | 1 | **0.50** | 3 | **2.38** | 25 | **8.46** | 0 | **0.00** | 14 | **7.47** | 0 | **0.00** |
| *A→T at 686* | *0* | ***0.00*** | *0* | ***0.00*** | *0* | ***0.00*** | *0* | ***0.00*** | *0* | ***0.00*** | *0* | ***0.00*** | *1* | ***0.50*** | *1* | ***0.79*** | *6* | ***2.03*** | *0* | ***0.00*** | *8* | ***4.27*** | *0* | ***0.00*** |
| *A→T at OS* | *2* | ***0.02*** | *0* | ***0.00*** | *1* | ***0.12*** | *1* | ***0.11*** | *1* | ***0.22*** | *0* | ***0.00*** | *0* | ***0.00*** | *2* | ***1.58*** | *19* | ***6.43*** | *0* | ***0.00*** | *6* | ***3.20*** | *0* | ***0.00*** |
| T→A | 1 | **0.01** | 3 | **0.04** | 4 | **0.47** | 0 | **0.00** | 0 | **0.00** | 4 | **0.81** | 2 | **1.00** | 4 | **3.17** | 3 | **1.02** | 7 | **2.69** | 2 | **1.07** | 6 | **4.47** |
| G→C | 1 | **0.01** | 0 | **0.00** | 0 | **0.00** | 0 | **0.00** | 0 | **0.00** | 0 | **0.00** | 0 | **0.00** | 1 | **0.79** | 0 | **0.00** | 0 | **0.00** | 0 | **0.00** | 4 | **2.98** |
| C→G | 0 | **0.00** | 0 | **0.00** | 1 | **0.12** | 1 | **0.11** | 0 | **0.00** | 0 | **0.00** | 0 | **0.00** | 0 | **0.00** | 0 | **0.00** | 0 | **0.00** | 0 | **0.00** | 0 | **0.00** |
| **Indels** | 9 | **0.10** | 8 | **0.11** | 8 | **0.95** | 4 | **0.45** | 7 | **1.54** | 6 | **1.22** | 4 | **2.01** | 2 | **1.58** | 2 | **0.68** | 0 | **0.00** | 11 | **5.87** | 5 | **3.73** |
| ΔA | **3** | **0.03** | **2** | **0.03** | **3** | **0.36** | **1** | **0.11** | **1** | **0.22** | **2** | **0.41** | **0** | **0.00** | **2** | **1.58** | **1** | **0.34** | **0** | **0.00** | **1** | **0.53** | **0** | **0.00** |
| *ΔA at 174-178* | *0* | ***0.00*** | *0* | ***0.00*** | *2* | ***0.24*** | *0* | ***0.00*** | *0* | ***0.00*** | *0* | ***0.00*** | *0* | ***0.00*** | *0* | ***0.00*** | 0 | ***0.00*** | 0 | ***0.00*** | 0 | ***0.00*** | 0 | ***0.00*** |
| *ΔA at OS* | *3* | ***0.03*** | *2* | ***0.03*** | *1* | ***0.12*** | *1* | ***0.11*** | *1* | ***0.22*** | *2* | ***0.41*** | *0* | ***0.00*** | *2* | ***1.58*** | 1 | ***0.34*** | 0 | ***0.00*** | 1 | ***0.53*** | 0 | ***0.00*** |
| ΔT | 1 | **0.01** | 2 | **0.03** | 2 | **0.24** | 0 | **0.00** | 5 | **1.10** | 1 | **0.20** | 0 | **0.00** | 0 | **0.00** | 0 | **0.00** | 0 | **0.00** | 0 | **0.00** | 1 | **0.75** |
| *ΔT at 201-205* | *0* | ***0.00*** | *0* | ***0.00*** | *1* | ***0.12*** | *0* | ***0.00*** | *0* | ***0.00*** | *0* | ***0.00*** | *0* | ***0.00*** | *0* | ***0.00*** | 0 | ***0.00*** | 0 | ***0.00*** | 0 | ***0.00*** | 0 | ***0.00*** |
| *ΔT at 255-260* | *0* | ***0.00*** | *1* | ***0.01*** | *1* | ***0.12*** | *0* | ***0.00*** | *2* | ***0.44*** | *0* | ***0.00*** | *0* | ***0.00*** | *0* | ***0.00*** | 0 | ***0.00*** | 0 | ***0.00*** | 0 | ***0.00*** | 1 | ***0.75*** |
| *ΔT at OS* | *1* | ***0.01*** | *1* | ***0.01*** | *0* | ***0.00*** | *0* | ***0.00*** | *3* | ***0.66*** | *1* | ***0.20*** | *0* | ***0.00*** | *0* | ***0.00*** | 0 | ***0.00*** | 0 | ***0.00*** | 0 | ***0.00*** | 0 | ***0.00*** |
| other single deletions | 0 | **0.00** | 0 | **0.00** | 0 | **0.00** | 0 | **0.00** | 1 | **0.22** | 1 | **0.20** | 3 | **1.51** | 0 | **0.00** | 0 | **0.00** | 0 | **0.00** | 2 | **1.07** | 3 | **2.24** |
| ≥2 deletions | 2 | **0.02** | 3 | **0.04** | 0 | **0.00** | 0 | **0.00** | 0 | **0.00** | 0 | **0.00** | 0 | **0.00** | 0 | **0.00** | 0 | **0.00** | 0 | **0.00** | 0 | **0.00** | 0 | **0.00** |
| single insertions | 3 | **0.03** | 1 | **0.01** | 3 | **0.36** | 3 | **0.34** | 0 | **0.00** | 2 | **0.41** | 1 | **0.50** | 0 | **0.00** | 1 | **0.34** | 0 | **0.00** | 8 | **4.27** | 1 | **0.75** |
| ≥2 insertions | 0 | **0.00** | 0 | **0.00** | 0 | **0.00** | 0 | **0.00** | 0 | 0.00 | 0 | **0.00** | 0 | **0.00** | 0 | **0.00** | 0 | 0.00 | 0 | **0.00** | 0 | **0.00** | 0 | **0.00** |
| **TOTAL** | 166 | **1.78** | 132 | **1.88** | 125 | **14.83** | 126 | **14.31** | 176 | **38.71** | 172 | **34.96** | 140 | **70.26** | 131 | **103.75** | 154 | **52.11** | 103 | **39.56** | 166 | **88.58** | 138 | **102.86** |
| **95% CI** | | 1.10<br>2.29 | | 1.32<br>2.87 | | 12.95<br>18.73 | | 10.72<br>21.90 | | 30.22<br>48.90 | | 26.92<br>52.80 | | 55.28<br>168.82 | | 90.97<br>158.86 | | 37.51<br>69.88 | | 15.67<br>61.67 | | 13.70<br>257.65 | | 62.25<br>173.87 |

[a] Number of events identified for given classes.
[b] Mutation rates [5-FOA$^R$×10$^{-6}$] for specific mutation types are shown in boldface.
[c] Specific hotspot positions in the *URA3* coding sequence are indicated.
[d] OS – Other Sites.

**SUPPLEMENTARY MATERIAL**

**Table S2.** The analysis of statistical significance for the mutation spectra is presented in Fig. 3. *p* values were calculated using Fisher's exact test.

| **T→C vs A→G**<br>**G→A vs C→T**<br>**G→T vs C→A** | *DPB2*<br>*POL3*<br>**OR2** | ***dpb2-100***<br>*POL3*<br>**OR1** | *DPB2*<br>***pol3-L612M***<br>**OR1** | ***dpb2-100***<br>***pol3-L612M***<br>**OR2** |
|---|---|---|---|---|
| *DPB2 POL3* **OR1** | **0.0007** | 0.3776 | **0.0005** | ND[a] |
| | **<0.0001** | 0.1733 | **<0.0001** | ND |
| | **0.0017** | 0.3702 | **0.0005** | ND |
| ***dpb2-100*** *POL3* **OR2** | **0.0012** | 0.5869 | ND | ND |
| | 0.2286 | **<0.0001** | ND | ND |
| | **<0.0001** | **<0.0001** | ND | ND |
| *DPB2* ***pol3-L612M*** **OR2** | **0.0008** | ND | **<0.0001** | **<0.0001** |
| | **<0.0001** | ND | **<0.0001** | **<0.0001** |
| | **<0.0001** | ND | **<0.0001** | **<0.0001** |
| ***dpb2-100 pol3-L612M*** **OR1** | ND | ND | >0.9999 | **<0.0001** |
| | ND | ND | **<0.0001** | **<0.0001** |
| | ND | ND | **<0.0001** | **<0.0001** |

[a] ND – not determined

**SUPPLEMENTARY MATERIAL**

**Table S3.** The analysis of statistical significance for data showing the contribution of substitutions at specific hotspots to the total mutagenesis is presented in Fig. 4. *p* values were calculated using Fisher's exact test.

| **T→C at 97**[a]<br>**C→T at 310**<br>**G→A at 764**<br>**G→T at 679/706** | *DPB2 POL3* **OR2** | ***dpb2-100*** *POL3* **OR1** | *DPB2* ***pol3-L612M*** **OR1** | ***dpb2-100 pol3-L612M*** **OR2** |
|---|---|---|---|---|
| *DPB2 POL3* **OR1** | **0.0175** [1][b] | **0.0006** ↓[c] | **0.0002** ↑ | ND[d] |
| | **0.0169** [2] | 0.3048 | **0.0253** ↓ | ND |
| | **<0.0001** [1] | **0.0407** ↓ | **<0.0001** ↑ | ND |
| | **<0.0001** [2] | 0.8534 | **<0.0001** ↓ | ND |
| ***dpb2-100*** *POL3* **OR2** | 0.3489 | 0.0600 | ND | ND |
| | 0.8899 | **<0.0001** [2] | ND | ND |
| | 0.7597 | **<0.0001** [1] | ND | ND |
| | **<0.0001** ↓ | **<0.0001** [2] | ND | ND |
| *DPB2* ***pol3-L612M*** **OR2** | 0.0508 | ND | **<0.0001** [1] | **<0.0001** ↑ |
| | **<0.0001** ↑ | ND | **<0.0001** [2] | **<0.0001** ↑ |
| | >0.9999 | ND | **<0.0001** [1] | **<0.0001** ↑ |
| | **<0.0001** ↓ | ND | **<0.0001** [2] | **0.0004** ↓ |
| ***dpb2-100 pol3-L612M*** **OR1** | ND | ND | **<0.0001** ↓ | **<0.0001** [1] |
| | ND | ND | **<0.0001** ↑ | **<0.0001** [2] |
| | ND | ND | **<0.0001** ↓ | **<0.0001** [1] |
| | ND | ND | **<0.0001** ↑ | **<0.0001** [2] |

[a] Substitutions at specific hotspots are color-coded.
[b] The *URA3* orientation with a higher contribution of substitutions at specific hotspots is shown in brackets: [1] – OR1. [2] – OR2.
[c] For *dpb2-100* and *pol3-L612M* mutants. an increase ↑ or decrease ↓ of substitutions at specific hotspots compared with the *DPB2 POL3* strain. is shown; for *dpb2-100 pol3-L612M* mutants. an increase ↑ or decrease ↓ of substitutions at specific hotspots compared with the *pol3-L612M* strain. is shown.
[d] ND – not determined

**Table S4.** The analysis of statistical significance for data showing the contribution of specific substitutions to the total mutagenesis is presented in Fig. 4. *p* values were calculated using Fisher's exact test.

| **T→C**[a]<br>**C→T**<br>**G→A**<br>**G→T** | *DPB2 POL3* **OR2** | ***dpb2-100*** *POL3* **OR1** | *DPB2* ***pol3-L612M*** **OR1** | ***dpb2-100 pol3-L612M*** **OR2** |
|---|---|---|---|---|
| *DPB2 POL3* **OR1** | **0.0006** [1][b] | **0.0294** ↓[c] | **<0.0001** ↑ | ND[d] |
| | 0.0928 | 0.0688 | **<0.0001** ↓ | ND |
| | **<0.0001** [1] | 0.7469 | **<0.0001** ↑ | ND |
| | **<0.0001** [2] | 0.8290 | **<0.0001** ↓ | ND |
| ***dpb2-100*** *POL3* **OR2** | **<0.0001** ↑ | **<0.0001** [2] | ND | ND |
| | **0.0160** ↑ | **<0.0001** [2] | ND | ND |
| | **0.0008** ↑ | **<0.0001** [1] | ND | ND |
| | **0.0007** ↓ | **<0.0001** [2] | ND | ND |
| *DPB2* ***pol3-L612M*** **OR2** | **0.0098** ↓ | ND | **<0.0001** [1] | **<0.0001** ↑ |
| | **<0.0001** ↑ | ND | **<0.0001** [2] | **<0.0001** ↓ |
| | 0.2544 | ND | **<0.0001** [1] | **<0.0001** ↑ |
| | **<0.0001** ↓ | ND | **<0.0001** [2] | **<0.0001** ↓ |
| ***dpb2-100 pol3-L612M*** **OR1** | ND | ND | **<0.0001** ↓ | **<0.0001** [1] |
| | ND | ND | **<0.0001** ↑ | **<0.0001** [2] |
| | ND | ND | **<0.0001** ↓ | **<0.0001** [1] |
| | ND | ND | **<0.0001** ↑ | **<0.0001** [2] |

[a] Specific substitution types are color-coded.
[b] The *URA3* orientation with a higher contribution of specific substitutions is shown in brackets: [1] – OR1. [2] – OR2.
[c] For *dpb2-100* and *pol3-L612M* mutants. an increase ↑ or decrease ↓ of specific substitutions compared with the *DPB2 POL3* strain. is shown; for *dpb2-100 pol3-L612M* mutants. an increase ↑ or decrease ↓ of specific substitutions compared with the *pol3-L612M* strain is shown.
[d] ND – not determined

**Table S5.** The analysis of statistical significance for the mutation spectra is presented in Fig. 6. *p* values were calculated using Fisher's exact test.

| **T→A vs A→T** | *DPB2 POL2* **OR2** | ***dpb2-100*** *POL2* **OR1** | *DPB2* ***pol2- M644G*** **OR1** | ***dpb2-100 pol2-M644G*** **OR2** |
|---|---|---|---|---|
| *DPB2 POL2* **OR1** | 0.1429 | 0.1251 | 0.2913 | ND[a] |
| ***dpb2-100*** *POL2* **OR2** | **0.0007** | **<0.0001** | ND | ND |
| *DPB2* ***pol2-M644G*** **OR2** | >0.9999 | ND | **<0.0001** | >0.9999 |
| ***dpb2-100 pol2-M644G*** **OR1** | ND | ND | 0.2691 | **<0.0001** |

[a] ND – not determined

**Table S6.** Yeast strains used in this work.

| Strain | Relevant genotype | Source |
|---|---|---|
| **YTAK001** | *agp1::URA3-OR1* | [43] |
| **Y485-3** | *agp1::URA3-OR1 rev3Δ DPB2 msh6Δ* | [44] |
| **Y485-4** | *agp1::URA3-OR1 rev3Δ DPB2 msh6Δ* | [44] |
| **Y773** | *agp1::URA3-OR1 rev3Δ dpb2-100 msh6Δ* | This work |
| **Y774** | *agp1::URA3-OR1 rev3Δ dpb2-100 msh6Δ* | This work |
| **Y771** | *agp1::URA3-OR1 rev3Δ dpb2-100 msh6Δ* | This work |
| **Y772** | *agp1::URA3-OR1 rev3Δ dpb2-100 msh6Δ* | This work |
| **YTAK002** | *agp1::URA3-OR2* | [43] |
| **Y486-2** | *agp1::URA3-OR2 rev3Δ DPB2 msh6Δ* | [44] |
| **Y486-5** | *agp1::URA3-OR2 rev3Δ DPB2 msh6Δ* | [44] |
| **Y776** | *agp1::URA3-OR2 rev3Δ dpb2-100 msh6Δ* | This work |
| **Y777** | *agp1::URA3-OR2 rev3Δ dpb2-100 msh6Δ* | This work |
| **Y778** | *agp1::URA3-OR2 rev3Δ dpb2-100 msh6Δ* | This work |
| **Y775** | *agp1::URA3-OR2 rev3Δ dpb2-100 msh6Δ* | This work |
| **Y779** | *agp1::URA3-OR2 rev3Δ dpb2-100 msh6Δ* | This work |
| **SNM12** | *pol3L612M agp1::URA3-OR1* | [90] |
| **Y491-3** | *pol3L612M agp1::URA3-OR1 rev3Δ DPB2 msh6Δ* | [44] |
| **Y492-1** | *pol3L612M agp1::URA3-OR1 rev3Δ DPB2 msh6Δ* | [44] |
| **Y783** | *pol3L612M agp1::URA3-OR1 rev3Δ dpb2-100 msh6Δ* | This work |
| **Y780** | *pol3L612M agp1::URA3-OR1 rev3Δ dpb2-100 msh6Δ* | This work |
| **Y781** | *pol3L612M agp1::URA3-OR1 rev3Δ dpb2-100 msh6Δ* | This work |
| **Y782** | *pol3L612M agp1::URA3-OR1 rev3Δ dpb2-100 msh6Δ* | This work |
| **SNM24** | *pol3L612M agp1::URA3-OR2* | [90] |
| **Y493-1** | *pol3L612M agp1::URA3-OR2 rev3Δ DPB2 msh6Δ* | [44] |
| **Y494-1** | *pol3L612M agp1::URA3-OR2 rev3Δ DPB2 msh6Δ* | [44] |
| **Y786** | *pol3L612M agp1::URA3-OR2 rev3Δ dpb2-100 msh6Δ* | This work |
| **Y787** | *pol3L612M agp1::URA3-OR2 rev3Δ dpb2-100 msh6Δ* | This work |
| **Y785** | *pol3L612M agp1::URA3-OR2 rev3Δ dpb2-100 msh6Δ* | This work |
| **Y784** | *pol3L612M agp1::URA3-OR2 rev3Δ dpb2-100 msh6Δ* | This work |
| **Y788** | *pol3L612M agp1::URA3-OR2 rev3Δ dpb2-100 msh6Δ* | This work |
| **SNM70** | *pol2M644G agp1::URA3-OR1* | [59] |
| **Y487-2** | *pol2M644G agp1::URA3-OR1 rev3Δ DPB2 msh6Δ* | [50] |
| **Y488-1** | *pol2M644G agp1::URA3-OR1 rev3Δ DPB2 msh6Δ* | [50] |
| **Y804** | *pol2M644G agp1::URA3-OR1 rev3Δ dpb2-100 msh6Δ* | This work |
| **Y805** | *pol2M644G agp1::URA3-OR1 rev3Δ dpb2-100 msh6Δ* | This work |
| **Y806** | *pol2M644G agp1::URA3-OR1 rev3Δ dpb2-100 msh6Δ* | This work |
| **Y807** | *pol2M644G agp1::URA3-OR1 rev3Δ dpb2-100 msh6Δ* | This work |
| **Y808** | *pol2M644G agp1::URA3-OR1 rev3Δ dpb2-100 msh6Δ* | This work |
| **Y809** | *pol2M644G agp1::URA3-OR1 rev3Δ dpb2-100 msh6Δ* | This work |
| **Y810** | *pol2M644G agp1::URA3-OR1 rev3Δ dpb2-100 msh6Δ* | This work |
| **SNM79** | *pol2M644G agp1::URA3-OR2* | [59] |
| **Y489-2** | *pol2M644G agp1::URA3-OR2 rev3Δ DPB2 msh6Δ* | [50] |
| **Y490-1** | *pol2M644G agp1::URA3-OR2 rev3Δ DPB2 msh6Δ* | [50] |
| **Y791** | *pol2M644G agp1::URA3-OR2 rev3Δ dpb2-100 msh6Δ* | This work |
| **Y792** | *pol2M644G agp1::URA3-OR2 rev3Δ dpb2-100 msh6Δ* | This work |
| **Y793** | *pol2M644G agp1::URA3-OR2 rev3Δ dpb2-100 msh6Δ* | This work |
| **Y794** | *pol2M644G agp1::URA3-OR2 rev3Δ dpb2-100 msh6Δ* | This work |
| **Y795** | *pol2M644G agp1::URA3-OR2 rev3Δ dpb2-100 msh6Δ* | This work |
| **SC228** | *MATa CAN1 his7-2 leu2-Δ::kanMX4 ura3-Δ trp1-289 ade2-1 lys2-ΔGG2899-2900 DPB2* | [25,39] |
| **SC234** | *MATa CAN1 his7-2 leu2-Δ::kanMX4 ura3-Δ trp1-289 ade2-1 lys2-ΔGG2899-2900 dpb2-100* | [25,39] |

**Table S7.** Primers used in this study.

| Primer | Sequence 5'-3' |
|---|---|
| Rev3_UPTEF | CAATACAAAACTACAAGTTGTGGCGAAATAAAATGTTTGGAAATGAGATCTGTTTAGCTTGCC |
| Rev3_DNTEF | ATAACTACTCATCATTTTGCGAGACATATCTGTGTCTAGATTATTCGAGCTCGTTTTCGACAC |
| msh6UTEF | CAGATAAGATTTTTTAATTGGAGCAACTAGTTAATTTTGACAAAGCCAATTTGAACTCCAAAAGATCTGTTTAGCTTGCC |
| msh6DTEF | CAACGACCAAAACTTTAAAAAAAATAAGTAAAAATCTTACATACATCGTAAATGAAAATATTCGAGCTCGTTTTCGACAC |
| Rev3-R4 | TGACCACTCACATGGCGCTTTG |
| Rev3A | AATTCTGCCAATCTATTTGATCTTG |
| nat1UO | ACCGGTAAGCCGTGTCGTCAAG |
| Rev3-F4 | AAAGGGCGAGCACAACTACTAC |
| Rev3D | CACCAGATAGAGTTTTGAACGAAAT |
| nat1DO | GCTTCGTGGTCGTCTCGTACTC |
| MSH6-UO | TAAAGTCGCTGGAGTAGG |
| msh6up2 | GAATCCTTGGAGGAAGAC |
| HPH-UO | ACAGACGTCGCGGTGAGTTCAG |
| MSH6-DO | TCAAGCACCATCCTCAAG |
| msh6dw2 | CCCATTCTTGCCCAAGATGC |
| HPH-DO | TCGCCGATAGTGGAAACCGACG |
| URA3F393 | AACGAAGGAAGGAGCACAGAC |
| URA3R412 | CCGAAATTCCTGGGTAATAAC |
| LEUBamF | AGTGGATCCACATACCTAATATTATTGCC |
| LEUMvaR | AAGGAGCATTCTGACAGAGTAAAATTCTTGAGGG |

**SUPPLEMENTARY MATERIAL**

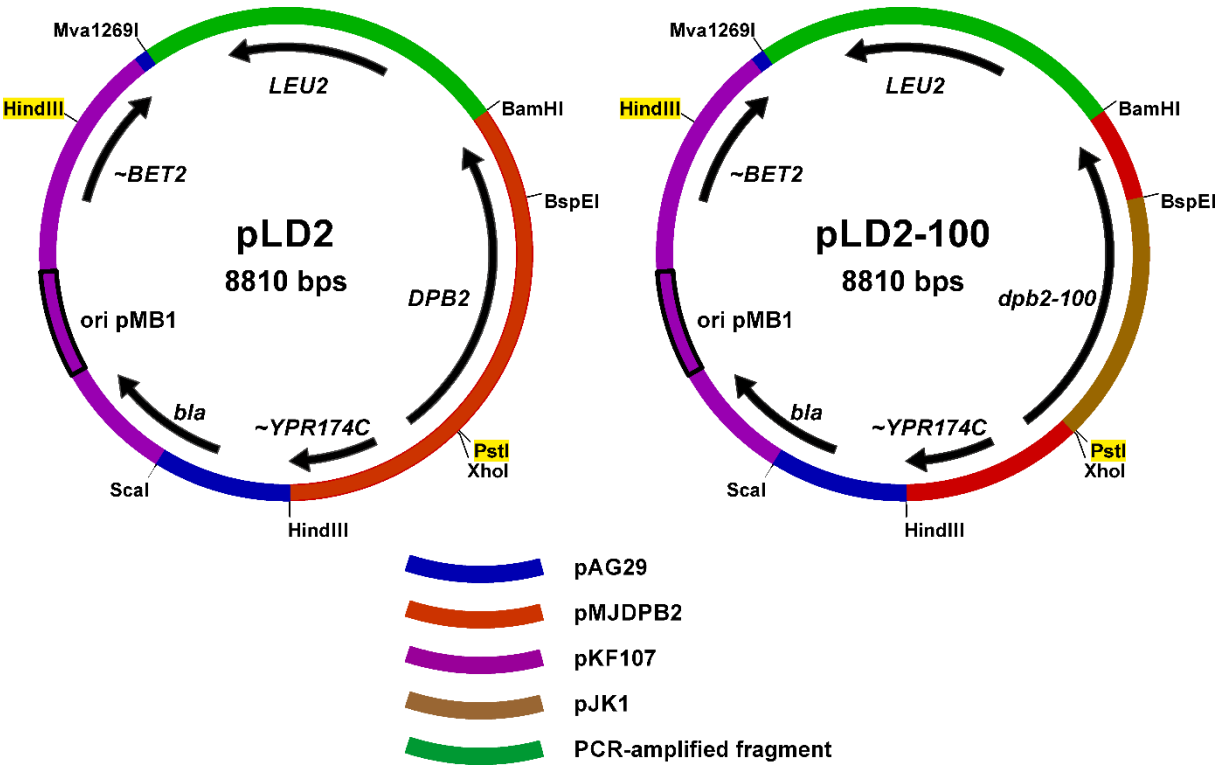


**Figure S1.** Maps of plasmid pLD2 and pLD2-100. For their construction. plasmids pAG29 [91]. pMJDPB2 [26]. pKF107 [25] and pJK1 [25] were used. The PCR fragment containing the *LEU2* gene was amplified using primers LEUBamF and LEUMvaR (Table 7). Restriction sites used to generate the gene cassette for yeast transformation are indicated by yellow backlight.

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