Supplementary Information for:

Genetic compatibility and extensibility of orthogonal replication

Alex A. Javanpour[†] and Chang C. Liu[*,†,‡,§]

[†]Department of Biomedical Engineering, University of California, Irvine, California 92697, United States

[‡]Department of Molecular Biology & Biochemistry, University of California, Irvine, California 92697, United States

[§]Department of Chemistry, University of California, Irvine, California 92697, United States

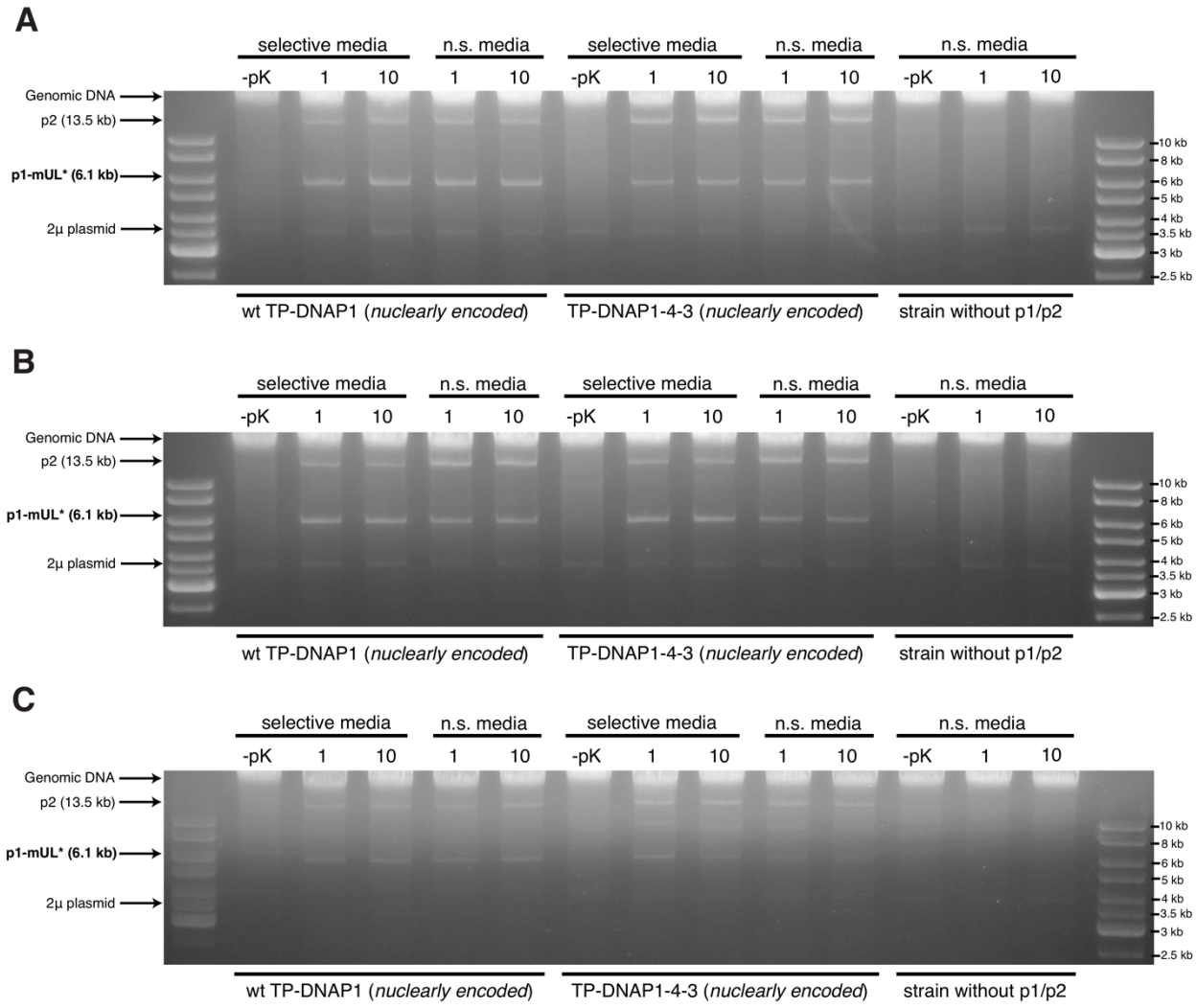

Figure S1— Stability of OrthoRep in CEN.PK2-1C (**A**), W303-1A (**B**), and BY4743 (**C**) strains. Agarose gel electrophoresis on DNA extracted from strains show the stability of OrthoRep (presence of p1-mUL*) over multiple cycles of passaging even without selection. Presence of p1-mUL* is observed after the first (1) and tenth (10) passage in both selective and non-selective (n.s.) media conditions in the presence of either a wt TP-DNAP1 or error-prone TP-DNAP1-4-3 expressed from a nuclear *CEN6/ARS4* plasmid. Parental strains without p1/p2 are shown as controls. The 2µ plasmid is native to the base strains CEN.PK2-1C, W303-1A, and BY4743. –pK conditions are controls referring to the lack of proteinase K treatment during DNA preparation; the lack of proteinase K treatment results in the lack of all p1/p2-derived bands because the terminal proteins on p1/p2 prevent migration into agarose gels as previously described.[2]

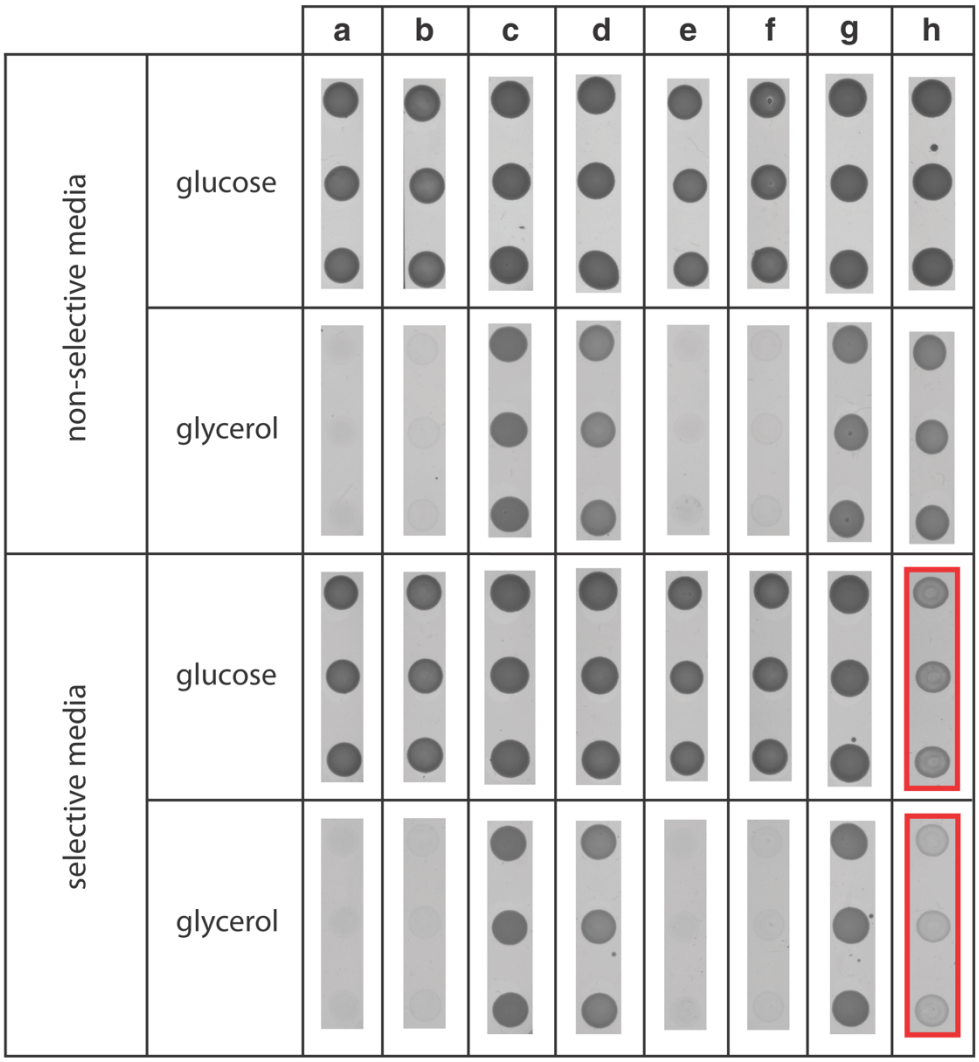

Figure S2—Ensuring that mitochondrial function hasn't changed in experiments outlined in **Figure 2**. Triplicates cultures from each condition in **Figure 2** (a-h) were spot plated (~10^6 cells) on media non-selective or selective for p1. Plates also either contained glucose or glycerol to assay for any respiration deficiency. Plates were incubated for ~4 days. For a, b, e, and f, lack of growth on glycerol shows the expected respiration deficiency of ρ^0 strains. For c, d, g, and h, growth on glycerol shows the expected respiration activity of ρ^+ strains. The red boxes highlight cells where the toxin is active in a ρ^+, which leads to instability of p1 and results in slower growth when selecting for p1-encoded genes.

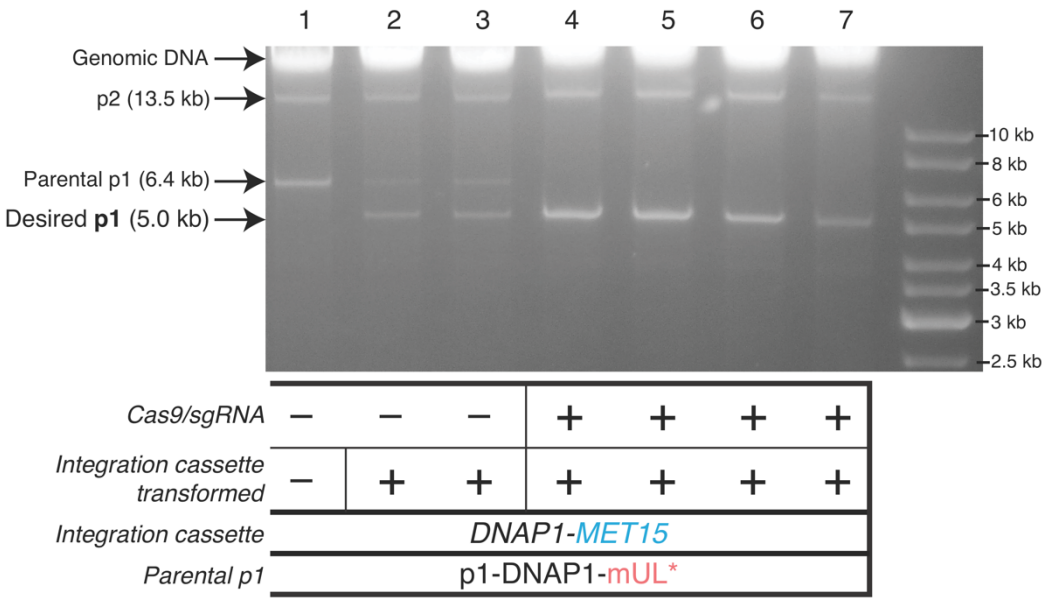

Figure S3— Integration of a model cassette (DNAP1-MET15) onto p1 in strains with p1-DNAP1-mUL* as the parental plasmid. This figure is exactly the same as Figure 3B, except it shows an experiment where the integration cassette genes and the parental p1 genes are swapped. Agarose gel electrophoresis on DNA extracted from cells after transformation and plating is shown (lanes 2-7). Lane 1 is the parental strain. Biological replicates from transformation without Cas9 induction (lanes 2 and 3) show both parental and desired p1 bands whereas biological replicates from a transformation with the integration cassette and concomitant induction of Cas9 (lanes 4-7) shows only the desired p1 band.

Table S1— Key plasmids used in this study. (See .xls file)

Table S2— Key strains used in this study. (See .xls file)

#	Name	Source	Parent Plasmid	Origin of replication (yeast, bacterial)	Selection Marker (yeast, bacterial)	Notes
1	AR-Ec318	Previous work (Ravikumar, 2018)	See previous work	*CEN6/ARS4, ColE1*	*HIS3, KanR*	*REV1* promoter > Recoded wt *TP-DNAP1*
2	AR-Ec632	Previous work (Ravikumar, 2018)	See previous work	*CEN6/ARS4, ColE1*	*HIS3, KanR*	*REV1* promoter > Recoded *TP-DNAP1 (L474W, L640Y, I777K, W814N)* with *HIS3* (TP-DNAP1-4-3)
3	AJ-Ec133	Addgene (#60847)	N/A	*2μ, ColE1*	*KanMX, KanR*	*tRNA TYR* promoter > *HDV* ribozyme + sgRNA > *SNR52* terminator and RNR2 promoter > *SpCAS9-SV40NLS-8XHIS* > *CYC1* terminator
4	AJ-Ec145	This Work	AJ-Ec133	*2μ, ColE1*	*KanMX, KanR*	contains 2 sgRNAs targetting *LEU2* locus (*GTAATAATGAAGATTATACA* and *TCAAGAATTTTACTCTGTCA*)
5	AJ-Ec170	This Work	AJ-Ec133	*2μ, ColE1*	*KanMX, KanR*	contains sgRNA targetting *URA3* (*GGGTCAACAGTATAGAACCG*)
6	AJ-Ec200	This Work	AJ-Ec133	*2μ, ColE1*	*KanMX, KanR*	contains sgRNA targetting *TRP1* (*GTCCATTGGTGAAAGTTTG*)
7	AJ-Ec137	This Work	AJ-Ec133	*2μ, ColE1*	*KanMX, KanR*	*tRNA TYR* promoter > *HDV* ribozyme + sgRNA > *SNR52* terminator and RNR2 promoter > *SpCAS9* > *CYC1* terminator
8	AJ-Ec138	This Work	AJ-Ec137	*2μ, ColE1*	*KanMX, KanR*	contains sgRNA targetting p1 ORF2 (*GCTGATTATACATATACAGA*)
9	AJ-Ec147	This Work	AJ-Ec137	*2μ, ColE1*	*KanMX, KanR*	contains sgRNA targetting *mKate2* (*TCTTCAAGTTGCAGATCAGG*)
10	AJ-Ec167	This Work	AJ-Ec147	*2μ, ColE1*	*KanMX, KanR*	*tRNA TYR* promoter > *HDV* ribozyme + *mKate2* sgRNA > *SNR52* terminator and *GAL2* promoter > *SpCAS9* > *CYC1* terminator
11	AJ-Ec168	This Work	AJ-Ec167	*2μ, ColE1*	*KanMX, KanR*	*tRNA TYR* promoter > *HDV* ribozyme + *MET15* sgRNA (*GCTAAGAAGTATCTATCTAA*) > *SNR52* terminator and *GAL2* promoter > *SpCAS9* > *CYC1* terminator
12	AJ-Ec180	This Work	N/A	N/A, *ColE1*	*URA3* (p1), *AmpR*	p1 recombination cassette that integrates *(p2ORF10 promoter > URA3)* to the 3' of *ORF4* to make p1-DNAP1-T/A-U
13	AR-Ec278	Previous Work (Ravikumar, 2014)	See previous work	N/A, *ColE1*	*URA3* (p1), *AmpR*	p1 recombination cassette that integrates *URA3* and *leu2** (*538C>T, 539T>G*) in place of ORFs 2-4 to create p1-DNAP1-UI*(TGA)
14	AJ-Ec154	This work	AR-Ec278	N/A, *ColE1*	*MET15* (p1), *AmpR*	p1 recombination cassette that integrates *(p1ORF2 promoter > MET15)* in place of ORFs 2-4 to make p1-DNAP1-MET15
15	AJ-Ec196	This work	AR-Ec505 (unpublished work)	N/A, *ColE1*	*URA3* (p1), *AmpR*	p1 recombination cassette that integrates *mKate2* , *URA3* , and *leu2* (538 T>C)* in place of ORFs 2-4 to make p1-DNAP1-mUl*(TAA)
16	AJ-Ec290	This work	AJ-Ec196	N/A, *ColE1*	*URA3* (p1), *AmpR*	p1 recombination cassette that integrates *mKate2* , *URA3* , and *K. lactis* genomic DNA in place of ORFs 2-4 to make p1-DNAP1-mU-kl14
17	AJ-Ec291	This work	AJ-Ec196	N/A, *ColE1*	*URA3* (p1), *AmpR*	p1 recombination cassette that integrates *mKate2* , *URA3* , and *K. lactis* genomic DNA in place of ORFs 2-4 to make p1-DNAP1-mU-kl18
18	AJ-Ec292	This work	AJ-Ec196	N/A, *ColE1*	*URA3* (p1), *AmpR*	p1 recombination cassette that integrates *mKate2* , *URA3* , and *K. lactis* genomic DNA in place of ORFs 2-4 to make p1-DNAP1-mU-kl22

Strain	Genotype	Parent Strain
AH22	*MAT* **a** *can1 his4-519 leu2-3, 112*	N/A
F102-2	*MAT* **a** *can1 his4-519 leu2-3, 112* ρ^0 + p1 + p2	N/A
AJ-Y86	*MAT* **a** *can1 leu2Δ0 ura3Δ0 his4-519* ρ^0 + p1 + p2	F102-2
AR-Y292	*MAT* **a** *can1 his3 leu2Δ0 ura3Δ0 HIS4* ρ^0 + p1 + p2	See previous work
AJ-Y119	*MAT* **a** can1 *his3 leu2Δ0 ura3Δ0 HIS4* ρ^0 + p1-DNAP1-TA-U [*p1ORF4::URA3*] + p2	AR-Y292
AJ-Y95	*MAT* **a** *can1 leu2Δ0 ura3Δ0 his4-519* ρ^0 + p1-DNAP1-mUL*(TAA) [*p1orf2Δ::URA3/mKate2/leu2(538C>T*)] + p2	AJ-Y86
AR-Y	*MAT* **a** *can1 his3 leu2Δ0 ura3Δ0 HIS4* ρ^0 + p1-mUL*(TAA) [*tpdnap1Δ::URA3/mKate2/leu2(538C>T)*] + p2 + AR-Ec318	See previous work
AR-Y	*MAT* **a** *can1 his3 leu2Δ0 ura3Δ0 HIS4* ρ^0 + p1-mUL*(TAA) [*tpdnap1Δ::URA3/mKate2/leu2(538C>T)*] + p2 + AR-Ec632	See previous work
AR-Y	*MAT* **a** *can1 his3 LEU2 URA3 HIS4* ρ^0 + p1-mW [*tpdnap1Δ::TRP1/mKate2*] + p2 + AR-Ec318	See previous work
AR-Y	*MAT* **a** *can1 his3 LEU2 URA3 HIS4* ρ^0 + p1-mW [*tpdnap1Δ::TRP1/mKate2*] + p2 + AR-Ec632	See previous work
BY4741	*MAT* **a** *his3 Δ 1 leu2Δ0 met15Δ0 ura3Δ0* ρ^+	N/A
AJ-Y90	*MAT* **a** *his3 Δ 1 leu2Δ0 met15Δ0 ura3Δ0* ρ^+ + p1-DNAP1-mUL*(TAA) [*p1orf2Δ::URA3/mKate2/leu2(538C>T*)] + p2	BY4741
AJ-Y91	*MAT* **a** *his3 Δ 1 leu2Δ0 met15Δ0 ura3Δ0* ρ^+ + p1-DNAP1-mUL*(TAA) *[p1orf2Δ::URA3/mKate2/leu2(538C>T)]* + p2 + AJ-Ec167	AJ-Y90
AJ-Y92	*MAT* **a** *his3 Δ 1 leu2Δ0 met15Δ0 ura3Δ0* ρ^+ + p1-DNAP1-MET15 [*p1orf2Δ::MET15*] + p2 + AJ-Ec168	AJ-Y91
AJ-Y170	*MAT* **a** *his3 Δ 1 leu2Δ0 met15Δ0 ura3Δ0* ρ^+ + p1-mUL*(TAA) [*tpdnap1Δ::URA3/mKate2/leu2(538C>T)*] + p2 + AR-Ec318	BY4741
AJ-Y171	*MAT* **a** *his3 Δ 1 leu2Δ0 met15Δ0 ura3Δ0* ρ^+ + p1-mUL*(TAA) [*tpdnap1Δ::URA3/mKate2/leu2(538C>T)*] + p2 + AR-Ec632	BY4741
AJ-Y190	*MAT* **a** *his3 Δ 1 LEU2 met15Δ0 URA3* ρ^+	BY4741
AJ-Y191	*MAT* **a** *his3 Δ 1 LEU2 met15Δ0 URA3* ρ^+ + p1-mW [*tpdnap1Δ::TRP1/mKate2*] + p2 + AR-Ec318	AJ-Y190
AJ-Y192	*MAT* **a** *his3 Δ 1 LEU2 met15Δ0 URA3* ρ^+ + p1-mW [*tpdnap1Δ::TRP1/mKate2*] + p2 + AR-Ec632	AJ-Y190
AJ-Y120	*MAT* **a** *his3 Δ 1 leu2Δ0 met15Δ0 ura3Δ0* ρ^+ + p1-DNAP1-TA-U [*p1met15Δ::URA3*] + p2	AJ-Y92
AJ-Y231	*MAT* **a** *his3 Δ 1 leu2Δ0 met15Δ0 ura3Δ0* ρ^+ + p1-DNAP1-mU-kl14 [*p1met15Δ::mKate2/URA3/* kl14] + p2	AJ-Y92
AJ-Y232	*MAT* **a** *his3 Δ 1 leu2Δ0 met15Δ0 ura3Δ0* ρ^+ + p1-DNAP1-mU-kl18 [*p1met15Δ::mKate2/URA3/* kl18] + p2	AJ-Y92
AJ-Y233	*MAT* **a** *his3 Δ 1 leu2Δ0 met15Δ0 ura3Δ0* ρ^+ + p1-DNAP1-mU-kl22 [*p1met15Δ::mKate2/URA3/* kl22] + p2	AJ-Y92
BY4743	*MAT* **a**/α *his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 LYS2/lys2Δ0 met15Δ0/MET15 ura3Δ0/ura3Δ0* ρ^+	N/A
AJ-Y172	*MAT* **a**/α *his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 LYS2/lys2Δ0 met15Δ0/MET15 ura3Δ0/ura3Δ0* ρ^+ + p1-mUL*(TAA) [*tpdnap1Δ::URA3/mKate2/leu2(538C>T)*] + p2 + AR-Ec318	BY4743
AJ-Y173	*MAT* **a**/α *his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 LYS2/lys2Δ0 met15Δ0/MET15 ura3Δ0/ura3Δ0* ρ^+ + p1-mUL*(TAA) [*tpdnap1Δ::URA3/mKate2/leu2(538C>T)*] + p2 + AR-Ec632	BY4743
AJ-Y193	*MAT* **a**/α *his3Δ1/his3Δ1 LEU2/LEU2 LYS2/lys2Δ0 met15Δ0/MET15 URA3::kanMX/ura3Δ0* ρ^+	BY4743
AJ-Y194	*MAT* **a**/α *his3Δ1/his3Δ1 LEU2/LEU2 LYS2/lys2Δ0 met15Δ0/MET15 URA3::kanMX/ura3Δ0* ρ^+ + p1-mW [*tpdnap1Δ::TRP1/mKate2*] + p2 + AR-Ec318	AJ-Y193
AJ-Y195	*MAT* **a**/α *his3Δ1/his3Δ1 LEU2/LEU2 LYS2/lys2Δ0 met15Δ0/MET15 URA3::kanMX/ura3Δ0* ρ^+ + p1-mW [*tpdnap1Δ::TRP1/mKate2*] + p2 + AR-Ec632	AJ-Y193
CEN.PK2-1C	*MAT* **a** *ura3-52 trp1-289 leu2-3,112 his3Δ1* ρ^+	N/A
AJ-Y073	*MAT* **a** *ura3Δ0 trp1-289 leu2Δ0 his3Δ1* ρ^+	CEN.PK2-1C
AJ-Y078	*MAT* **a** *ura3Δ0 TRP1 leu2Δ0 his3Δ1* ρ^+	AJ-Y073
AJ-Y174	*MAT* **a** *ura3Δ0 TRP1 leu2Δ0 his3Δ1* ρ^+ + p1-mUL*(TAA) [*tpdnap1Δ::URA3/mKate2/leu2(538C>T)*] + p2 + AR-Ec318	AJ-Y073
AJ-Y175	*MAT* **a** *ura3Δ0 TRP1 leu2Δ0 his3Δ1* ρ^+ + p1-mUL*(TAA) [*tpdnap1Δ::URA3/mKate2/leu2(538C>T)*] + p2 + AR-Ec632	AJ-Y073
AJ-Y196	*MAT* **a** *URA3 trp1Δ0 LEU2 his3Δ1* ρ^+	CEN.PK2-1C
AJ-Y197	*MAT* **a** *URA3 trp1Δ0 LEU2 his3Δ1* ρ^+ + p1-mW [*tpdnap1Δ::TRP1/mKate2*] + p2 + AR-Ec318	AJ-Y196
AJ-Y198	*MAT* **a** *URA3 trp1Δ0 LEU2 his3Δ1* ρ^+ + p1-mW [*tpdnap1Δ::TRP1/mKate2*] + p2 + AR-Ec632	AJ-Y196
W303-1A	*MAT* **a** *ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100* ρ^+	N/A
AJ-Y074	*MAT* **a** *ade2-1 his3-11,15 leu2Δ0 trp1-1 ura3Δ0 can1-100* ρ^+	W303-1A
AJ-Y079	*MAT* **a** *ade2-1 his3-11,15 leu2Δ0 TRP1 ura3Δ0 can1-100* ρ^+	AJ-Y074
AJ-Y176	*MAT* **a** *ade2-1 his3-11,15 leu2Δ0 TRP1 ura3Δ0 can1-100* ρ^+ + p1-mUL*(TAA) [*tpdnap1Δ::URA3/mKate2/leu2(538C>T)*] + p2 + AR-Ec318	AJ-Y074
AJ-Y177	*MAT* **a** *ade2-1 his3-11,15 leu2Δ0 TRP1 ura3Δ0 can1-100* ρ^+ + p1-mUL*(TAA) [*tpdnap1Δ::URA3/mKate2/leu2(538C>T)*] + p2 + AR-Ec632	AJ-Y074
AJ-Y199	*MAT* **a** *ade2-1 his3-11,15 LEU2 trp1-1 URA3 can1-100* ρ^+	W303-1A
AJ-Y200	*MAT* **a** *ade2-1 his3-11,15 LEU2 trp1-1 URA3 can1-100* ρ^+ + p1-mW [*tpdnap1Δ::TRP1/mKate2*] + p2 + AR-Ec318	AJ-Y199
AJ-Y201	*MAT* **a** *ade2-1 his3-11,15 LEU2 trp1-1 URA3 can1-100* ρ^+ + p1-mW [*tpdnap1Δ::TRP1/mKate2*] + p2 + AR-Ec632	AJ-Y199