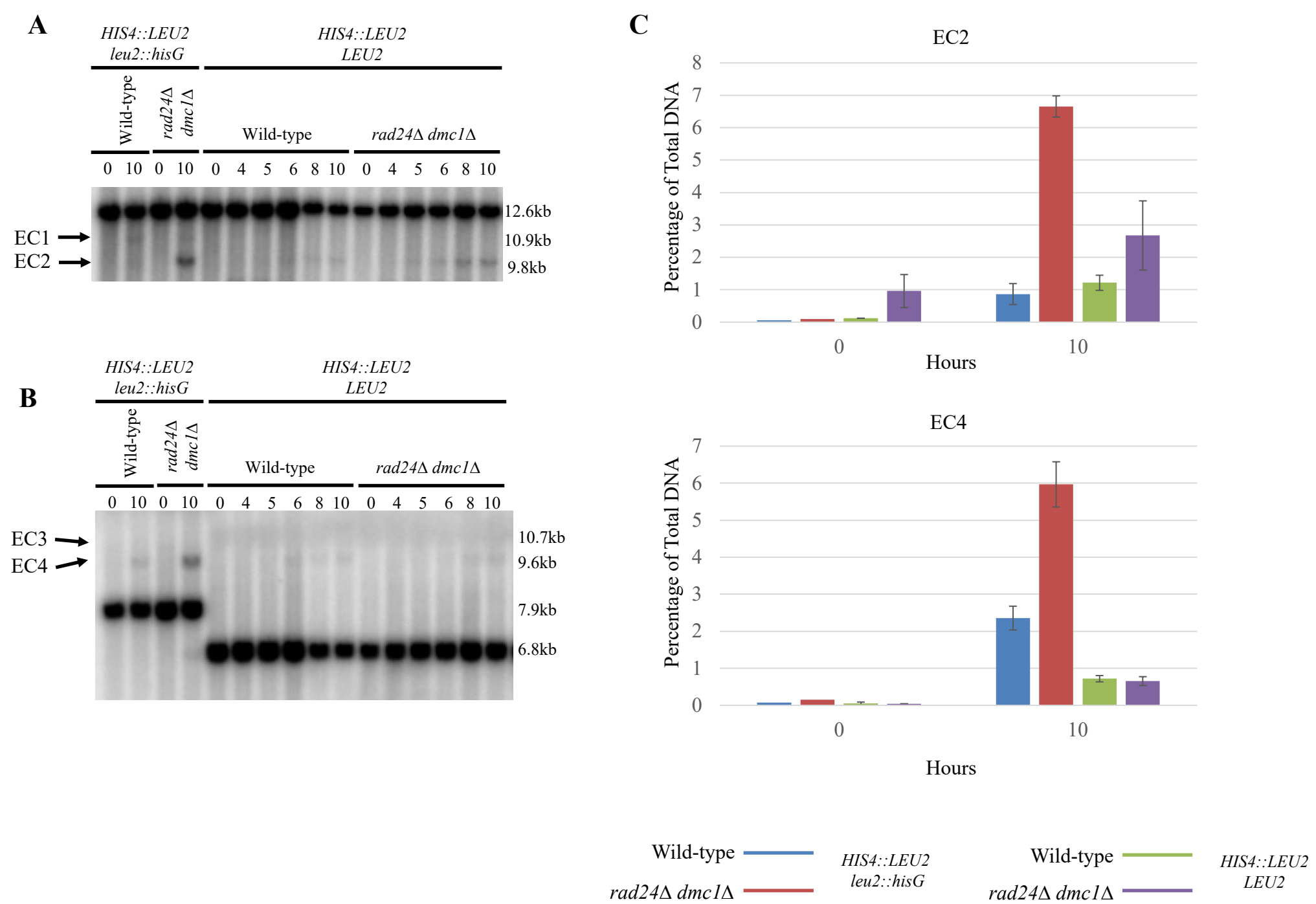
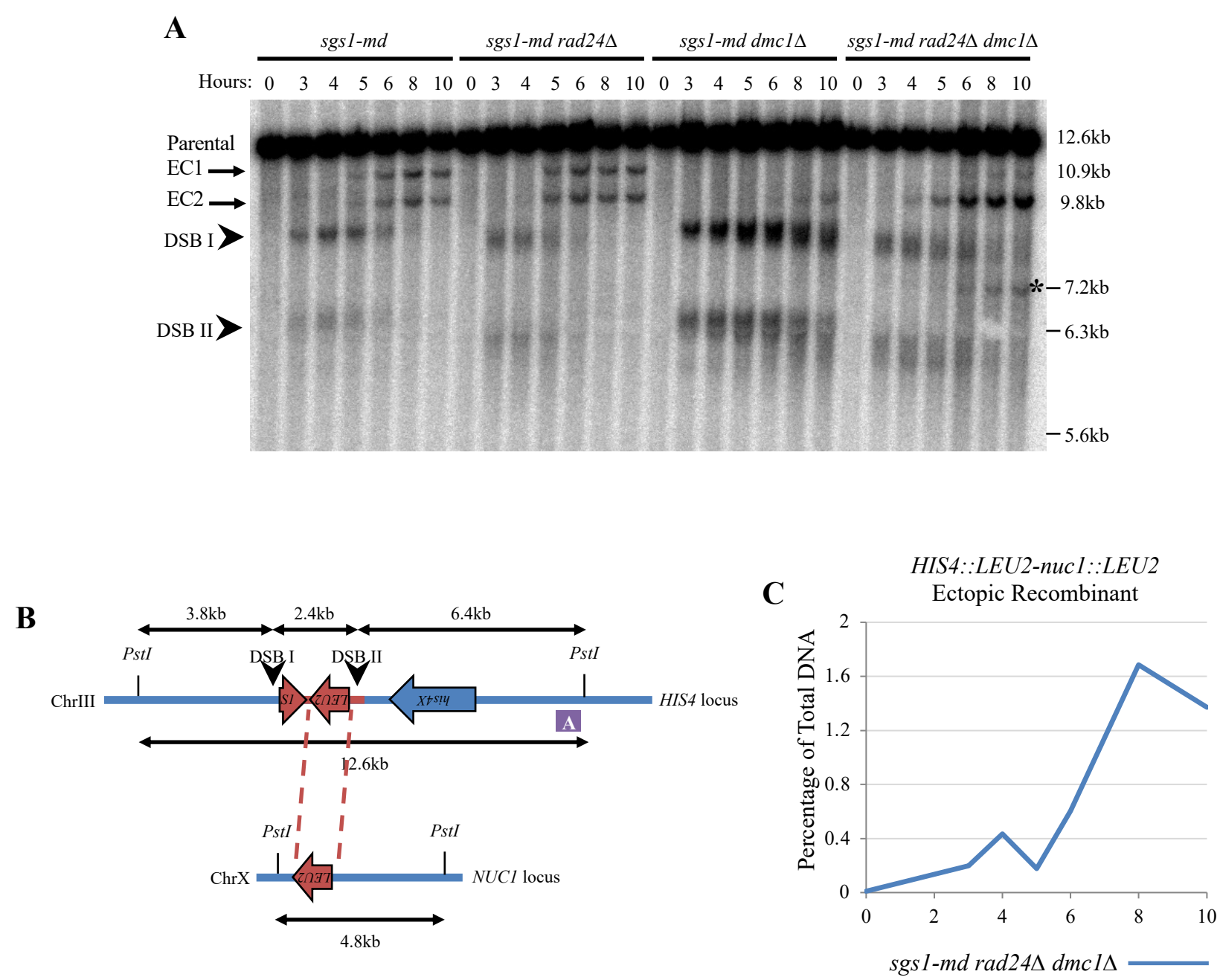


Supplemental Figure 1: Ectopic recombinants 1 and 3 are dependent upon *leu2::hisG*



**Supplemental Figure 1: Ectopic recombinants 1 and 3 are dependent upon *leu2::hisG*.** (A) Southern blot using *HIS4 LH* probe (probe A) and (B) *LEU2* probe (probe B) showing ectopic recombinants (arrows). (C) Quantification of ectopic recombinant levels from (A) and (B) with average ectopic recombinants signal plotted with standard deviation.

Supplemental Figure 2: Ectopic recombinants can form between *HIS4::LEU2* and *nuc1::LEU2*



**Supplemental Figure 2: Ectopic recombinants can form between *HIS4::LEU2* and *nuc1::LEU2*.** (A) Southern blot shown in figure 2 using *HIS4 LH* probe (probe A, purple box), with DSBs and ectopic recombinants. Asterisk indicates additional ectopic recombinant product. (B) Schematic of the *HIS4::LEU2* hotspot on chromosome III (top) and the *NUC1::LEU2* locus on chromosome X (bottom). Dotted lines indicate homology. (C) Quantification of ectopic recombinant shown in (A).

## Supplemental Table 1: Summary of mutant phenotypes

Genotype	Phenotype	Recombinant Types	Average Total Ectopic Recombinants (10 hours)	DSB Resection	Checkpoint Function	Inter-homologue Bias	Ectopic Repair Pathways
Wild-type		EC1, EC2, EC3, EC4	5.48%	Normal	Active	Active	HR, RD-SSA, RI-SSA, RD-BIR, RI-BIR
<i>rad24Δ</i>		EC1, EC2, EC3, EC4	9.69%	Hyper-resected	Absent	Defective	HR, RD-SSA, RI-SSA, RD-BIR, RI-BIR
<i>dmc1Δ</i>		EC2, EC4	1.96%	Continued Resection	Arrested	Active	(HR), (RD-SSA), (RD-BIR), RI-SSA, RI-BIR
<i>rad24Δ dmc1Δ</i>		EC2, EC4	13.1%	Hyper-resected	Absent	Defective	(HR), (RD-SSA), (RD-BIR), RI-SSA, RI-BIR
<i>sgs1-md</i>		EC1, EC2, EC3, EC4	17.11%	Normal	Active	Defective	HR, RD-SSA, RI-SSA, RD-BIR, RI-BIR
<i>sgs1-md rad24Δ</i>		EC1, EC2, EC3, EC4	21.24%	Hyper-resected	Absent	Defective	HR, RD-SSA, RI-SSA, RD-BIR, RI-BIR
<i>sgs1-md dmc1Δ</i>		EC2, EC4	5.78%	Continued Resection	Arrested	Defective	(HR), (RD-SSA), (RD-BIR), RI-SSA, RI-BIR
<i>sgs1-md rad24Δ dmc1Δ</i>		(EC1), EC2, EC4	16.22%	Hyper-resected	Absent	Defective	(HR), (RD-SSA), (RD-BIR), RI-SSA, RI-BIR
<i>rad51Δ dmc1Δ</i>		EC2, EC4	9.04%	Continued Resection	Arrested	Active	RI-SSA, RI-BIR
<i>rad24Δ rad51Δ dmc1Δ</i>		EC2, EC4	8.01%	Hyper-resected	Absent	Defective	RI-SSA, RI-BIR

**Supplemental Table 1: Summary of Mutant Phenotypes:** Genotypes of strains investigated in this study with predominant ectopic recombinant types observed. Average ectopic recombinant levels from figures 1, 2 and 5 at 10 hours. Resection phenotypes as described in (36,37,40,41). Checkpoint function as described from (36,37). Inter-homologue bias as described in this study and (18,24). Potential ectopic recombinant repair pathways with brackets indicating unknown function.

**Supplemental Table 2: Genotypes of strains used in this study**

<b>Strain</b>	<b>Number</b>	<b>Genotype</b>
wildtype	MJ6	<i>ho::LYS2'</i> , <i>lys2'</i> , <i>ura3'</i> , <i>arg4-nsp'</i> , <i>leu2::hisG'</i> , <i>his4X::LEU2'</i> , <i>nuc1::LEU2'</i>
<i>sae2Δ</i>	MJ315	<i>ho::LYS2'</i> , <i>lys2'</i> , <i>ura3'</i> , <i>arg4-nsp'</i> , <i>leu2::hisG'</i> , <i>his4X::LEU2'</i> , <i>nuc1::LEU2'</i> , <i>sae2Δ::KanMX'</i>
<i>rad24Δ</i>	SG150	<i>ho::LYS2'</i> , <i>lys2'</i> , <i>ura3'</i> , <i>arg4-nsp'</i> , <i>leu2::hisG'</i> , <i>his4X::LEU2'</i> , <i>nuc1::LEU2'</i> , <i>rad24Δ::hpHMX4'</i>
<i>dmc1Δ</i>	SG147	<i>ho::LYS2'</i> , <i>lys2'</i> , <i>ura3'</i> , <i>arg4-nsp'</i> , <i>leu2::hisG'</i> , <i>his4X::LEU2'</i> , <i>nuc1::LEU2'</i> , <i>dmc1Δ::LEU2'</i>
<i>rad24Δ dmc1Δ</i>	SG146	<i>ho::LYS2'</i> , <i>lys2'</i> , <i>ura3'</i> , <i>arg4-nsp'</i> , <i>leu2::hisG'</i> , <i>his4X::LEU2'</i> , <i>nuc1::LEU2'</i> , <i>dmc1Δ::LEU2'</i> , <i>rad24Δ::hpHMX4'</i>
wildtype	MJ1395	<i>ho::LYS2'</i> , <i>lys2'</i> , <i>ura3'</i> , <i>arg4-nsp'</i> , <i>his4X::LEU2'</i>
<i>rad24Δ dmc1Δ</i>	MJ1398	<i>ho::LYS2'</i> , <i>lys2'</i> , <i>ura3'</i> , <i>arg4-nsp'</i> , <i>his4X::LEU2'</i> , <i>dmc1Δ::LEU2'</i> , <i>rad24Δ::hpHMX4'</i>
<i>sgs1-md</i>	SG193	<i>ho::LYS2'</i> , <i>lys2'</i> , <i>ura3'</i> , <i>arg4-nsp-?'</i> , <i>leu2::hisG'</i> , <i>his4X::LEU2'</i> , <i>nuc1::LEU2'</i> , <i>natMX::pCLB2::3HA::SGS1'</i>
<i>sgs1-md rad24Δ</i>	SG153	<i>ho::LYS2'</i> , <i>lys2'</i> , <i>ura3'</i> , <i>arg4-nsp-?'</i> , <i>leu2::hisG'</i> , <i>his4X::LEU2'</i> , <i>nuc1::LEU2'</i> , <i>rad24Δ::hpHMX4'</i> , <i>natMX::pCLB2::3HA::SGS1'</i>
<i>sgs1-md dmc1Δ</i>	SG547	<i>ho::LYS2'</i> , <i>lys2'</i> , <i>ura3'</i> , <i>arg4-nsp'</i> , <i>leu2::hisG'</i> , <i>his4X::LEU2'</i> , <i>nuc1::LEU2'</i> , <i>natMX::pCLB2::3HA::SGS1'</i> , <i>dmc1Δ::LEU2'</i>
<i>sgs1-md rad24Δ dmc1Δ</i>	SG223	<i>ho::LYS2'</i> , <i>lys2'</i> , <i>ura3'</i> , <i>arg4-nsp-?'</i> , <i>leu2::hisG'</i> , <i>his4X::LEU2'</i> , <i>nuc1::LEU2'</i> , <i>dmc1Δ::LEU2'</i> , <i>rad24Δ::hpHMX4'</i> , <i>natMX::pCLB2::3HA::SGS1'</i>
<i>rad51Δ dmc1Δ</i>	SG271	<i>ho::LYS2'</i> , <i>lys2'</i> , <i>ura3'</i> , <i>arg4-?'</i> , <i>leu2::hisG'</i> , <i>his4X::LEU2'</i> , <i>nuc1::LEU2'</i> , <i>rad51Δ::hisG-URA3-hisG'</i> , <i>dmc1Δ::HphMX'</i>
<i>rad24Δ rad51Δ dmc1Δ</i>	SG243	<i>ho::hisG'</i> , <i>lys2'</i> , <i>ura3'</i> , <i>arg4-nsp'</i> , <i>leu2::hisG'</i> , <i>his4X::LEU2'</i> , <i>nuc1::LEU2'</i> , <i>dmc1Δ::LEU2'</i> , <i>rad24Δ::hpHMX4'</i> , <i>rad51Δ::hisG-URA3-hisG'</i>
<i>PRD1::pMN164/PRD1</i>	MJ1140	<i>ho::LYS2'</i> , <i>lys2'</i> , <i>ura3'</i> , <i>arg4-nsp'</i> , <i>leu2::hisG'</i> , <i>his4X::LEU2'</i> , <i>nuc1::LEU2'</i> , <i>PRD1::pMN164-URA3/PRD1</i>
<i>PRD1::pMN164/PRD1 rad24Δ</i>	MJ1143	<i>ho::LYS2'</i> , <i>lys2'</i> , <i>ura3'</i> , <i>arg4-nsp'</i> , <i>leu2::hisG'</i> , <i>his4X::LEU2'</i> , <i>nuc1::LEU2'</i> , <i>PRD1::pMN164-URA3/PRD1</i> , <i>rad24Δ::Hyg'</i>
<i>PRD1::pMN164/PRD1 sgs1-md</i>	MJ1146	<i>ho::LYS2'</i> , <i>lys2'</i> , <i>ura3'</i> , <i>arg4-nsp'</i> , <i>leu2::hisG'</i> , <i>his4X::LEU2'</i> , <i>nuc1::LEU2'</i> , <i>PRD1::pMN164-URA3/PRD1</i> , <i>natMX::pCLB2::3HA::SGS1'</i>
<i>PRD1::pMN164/PRD1 sgs1-md rad24Δ</i>	MJ1149	<i>ho::LYS2'</i> , <i>lys2'</i> , <i>ura3'</i> , <i>arg4-nsp'</i> , <i>leu2::hisG'</i> , <i>his4X::LEU2'</i> , <i>nuc1::LEU2'</i> , <i>PRD1::pMN164-URA3/PRD1</i> , <i>rad24Δ::Hyg'</i> , <i>natMX::pCLB2::3HA::SGS1'</i>