

Appendix PDF

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Appendix Table S1. Analysis of the allelic recombination products. The percentage of indicated products among tested colonies. Number of tested colonies is shown in parentheses.

	"BIR"	Gene conversion	Cross-over	Chromosome loss	Others
WT (597)	0.5 (3)	81.37 (485.75)	16.75 (100)	0.67 (4)	0.71 (4.25)
<i>rad59</i> (623)	2.81 (17.5)	80.42 (501)	15.41 (96)	0.1 (0.75)	1.26 (7.75)
<i>rad52-R70A</i> (509)	6.73 (34.25)	72.94 (371.25)	15.32 (78)	3.14 (16)	1.87 (9.5)
<i>rad59 rad52-R70A</i> (571)	8.84 (50.5)	64.58 (368.75)	16.46 (94)	6.79 (38.75)	3.33 (19)
<i>pif1</i> (491)	0.41 (2)	82.99 (407.5)	15.07 (74)	0.71 (3.5)	0.81 (4)
<i>pif1 rad52-R70A</i> (755)	1.03 (7.75)	81.09 (612.25)	16.42 (124)	0.73 (5.5)	0.73 (5.5)
<i>mre11</i> (510)	7.84 (40)	64.8 (330.5)	17.25 (88)	6.18 (31.5)	3.92 (20)
<i>mph1</i> (402)	0.62 (2.5)	76 (305.5)	22.39 (90)	0 (0)	1 (4)
<i>mph1 rad59</i> (664)	8.06 (53.5)	69.62 (462.25)	17.17 (114)	0.15 (1)	5.01 (33.25)
<i>mph1 rad52-R70A</i> (604)	10.35 (62.5)	63.82 (385.5)	17.22 (104)	1.9 (11.5)	6.71 (40.5)
<i>mph1 rad59 rad52-R70A</i> (541)	11.65 (63)	63.86 (345.5)	13.31 (72)	7.95 (43)	3.23 (17.5)

Appendix Table S2: Yeast strains used in this study.

Strain name	Parental Strain	Genotype	Source
A. Original strains			
YAM033		<i>ho ade3::GAL10::HO HMLα-inc MATa hmr::ADE1 bar1::ADE3 nej1::KANMX ade1 leu2,3-112 trp1::hisG ura3-52 thr4 lys5</i>	(Mehta et. al., 2017)
JKM139		<i>MATa ho hml::ADE1 hmr::ADE1 ade1 leu2-3,-112 lys5 trp1::hisG ura3-52 lys5 ade3::GAL10::HO</i>	(Lee et. al, 1998)
JKM179		<i>MATα ho hml::ADE1 hmr::ADE1 ade1 leu2-3,-112 lys5 trp1::hisG ura3-52 lys5 ade3::GAL10::HO</i>	(Lee et. al, 1998)
B. H-150 system			
YAM095		<i>ho ade3::GAL10::HO HMLα-inc MATa hmr::ADE1 bar1::ADE3 nej1::KANMX ade1 leu2,3-112 trp1::hisG ura3-52 thr4 lys5, WΔ, MAT XΔ150bp::Cg-TRP1</i>	(Mehta et. al., 2017)
NP493	YAM095	<i>rad59::NATMX</i>	This study
NP532	YAM095	<i>rad52-R70A</i>	This study
NP534	YAM095	<i>rad59::NATMX rad52-R70A</i>	This study
NP817	YAM095	<i>sir2::HPHMX</i>	This study
NP852	YAM095	<i>yku70::NATMX</i>	This study
NP853	YAM095	<i>mre11::HPHMX</i>	This study
NP857	YAM095	<i>yku70::NATMX mre11::HPHMX</i>	This study
NP856	YAM095	<i>rad51::LEU2MX</i>	This study
NP868	YAM095	<i>rad52::HPHMX</i>	This study
NP859	YAM095	<i>dot1::NATMX</i>	This study
C. H-150 no gap system			
NP764	NP578	<i>WΔ MAT XΔ Yα150bp::Cg-TRP1</i>	This study
NP743	NP764	<i>rad59::NATMX</i>	This study
NP775	NP764	<i>rad52-R70A</i>	This study
NP751	NP764	<i>rad59::NATMX rad52-R70A</i>	This study
NP829	NP764	<i>sir2::HPHMX</i>	This study
NP843	NP764	<i>mre11::HPHMX</i>	This study
NP870	NP764	<i>yku70::NATMX</i>	This study
NP873	NP764	<i>yku70::NATMX mre11::HPHMX</i>	This study
D. H-1400 system			
NP575	YAM033	<i>rad59::NATMX</i>	This study
NP576	YAM033	<i>rad52-R70A</i>	This study
NP577	YAM033	<i>rad59::NATMX rad52-R70A</i>	This study
NP664	YAM033	<i>mph1::HPHMX</i>	This study
NP665	YAM033	<i>mph1::HPHMX rad59::NATMX</i>	This study
NP696	YAM033	<i>mph1::HPHMX rad52-R70A</i>	This study
NP697	YAM033	<i>mph1::HPHMX rad59::NATMX rad5-2R70A</i>	This study

NP818	YAM033	<i>sir2::HPHMX</i>	This study
E. H-2100 system			
NP578	YAM033	<i>ho ade3::GAL10::HO HMLα-inc MATα hmr::ADE1 bar1::ADE3 nej1::KANMX ade1 leu2,3-112 trp1::hisG ura3-52 thr4 lys5</i>	This study
NP579	NP578	<i>rad59::NATMX</i>	This study
NP580	NP578	<i>rad52-R70A</i>	This study
F. Allelic Recombination			
NP633	YAM033	<i>MATα-inc HMLα-inc hmr::ADE1 bar1::ADE3 ade1 leu2,3-112 trp1::hisG ura3-52 thr4 lys5 ade3::GAL10::HO</i>	This study
NP676	JKM139	<i>kiTRP</i> inserted at location 17130 <i>LEU2</i> at 296670 on Chr III	This study
NP643	NP633	<i>KANMX</i> inserted at location 296670 on Chr III	This study
NP677	NP676	<i>rad59::NATMX</i>	This study
NP681	NP676	<i>rad52-R70A</i>	This study
NP682	NP676	<i>rad59::NATMX rad52-R70A</i>	This study
NP645	NP643	<i>rad59::NATMX</i>	This study
NP646	NP643	<i>rad52-R70A</i>	This study
NP647	NP643	<i>rad59::NATMX rad52-R70A</i>	This study
NP698	NP676 x NP643	<i>MATα/MATα-inc</i>	This study
NP699	NP677 x NP645	<i>rad59::NATMX/ rad59::NATMX</i>	This study
NP700	NP681 x NP646	<i>rad52-R70A/ rad52-R70A</i>	This study
NP701	NP682 x NP647	<i>rad59::NATMX/rad59::NATMX rad52-R70A/rad52-R70A</i>	This study
NP729	NP676	<i>ura3Δ</i>	This study
NP728	NP643	<i>ura3Δ</i>	This study
NP769	NP677	<i>ura3Δ</i>	This study
NP712	NP645	<i>ura3Δ</i>	This study
NP714	NP681	<i>ura3Δ</i>	This study
NP713	NP646	<i>ura3Δ</i>	This study
NP794	NP729	<i>pif1::HPHMX</i>	This study
NP795	NP728	<i>pif1::HPHMX</i>	This study
NP815	NP728 x NP729	<i>pif1::HPHMX/ pif1::HPHMX</i>	This study
NP798	NP714	<i>pif1::HPHMX</i>	This study
NP811	NP713	<i>pif1::HPHMX</i>	This study
NP816	NP798 x NP811	<i>pif1::HPHMX/pif1::HPHMX rad52-R70A/rad52-R70A</i>	This study
NP693	NP676	<i>mph1::HPHMX</i>	This study
NP662	NP643	<i>mph1::HPHMX</i>	This study
NP702	NP662 x NP693	<i>mph1::HPHMX/ mph1::HPHMX</i>	This study
NP694	NP677	<i>mph1::HPHMX</i>	This study
NP671	NP645	<i>mph1::HPHMX</i>	This study

NP703	NP694 x NP671	<i>mph1::HPHMX/mph1::HPHMX rad59::NATMX/rad59::NATMX</i>	This study
NP710	NP681	<i>mph1::HPHMX</i>	This study
NP673	NP646	<i>mph1::HPHMX</i>	This study
NP705	NP673 x NP710	<i>mph1::HPHMX/mph1::HPHMX rad52-R70A/rad52-R70A</i>	This study
NP695	NP682	<i>mph1::HPHMX</i>	This study
NP674	NP647	<i>mph1::HPHMX</i>	This study
NP706	NP695 x NP674	<i>mph1::HPHMX/mph1::HPHMX rad59::NATMX/rad59::NATMX rad52-R70A/rad52-R70A</i>	This study
NP814	NP729	<i>mre11::HPHMX</i>	This study
NP835	NP728	<i>mre11::HPHMX</i>	This study
NP841	NP814 x NP835	<i>mre11::HPHMX/mre11::HPHMX</i>	This study
G. Mutation analysis			
NP754	NP728	<i>HYG-ura3-29</i> inserted 16kb away from <i>MATα-inc</i>	This study
NP783	NP712	<i>HYG-ura3-29</i> inserted 16kb away from <i>MATα-inc</i>	This study
NP733	NP713	<i>HYG-ura3-29</i> inserted 16kb away from <i>MATα-inc</i>	This study
NP757	NP729 x NP754		This study
NP786	NP769 x NP783		This study
NP755	NP733 x NP714		This study
H. H-0 system			
NP477	YAM033	<i>WΔ, MAT XΔ::Cg-TRP1</i>	This study
NP494	YAM033	<i>rad59::NATMX</i>	This study
NP531	YAM033	<i>rad52-R70A</i>	This study
NP625	YAM033	<i>rad59::NATMX rad52-R70A</i>	This study
NP717	NP477	<i>mph1::HPHMX</i>	This study
NP718	NP477	<i>mph1::HPHMX rad5-2R70A</i>	This study
NP828	NP477	<i>sir2::HPHMX</i>	This study
I. HR-0 system			
NP582		<i>ho ade3::GAL10::HO HMRA-inc MATα hml::ADE1 bar1::ADE3 ade1 leu2,3-112 trp1::hisG ura3-52 thr4 lys5</i>	This study
NP860	NP582	<i>WΔ, MAT XΔ::Cg-TRP1</i>	This study
J. Disomic BIR system			
AM1003		<i>MATα-LEU2-tel/MATα-inc ade1 met13 ura3 leu2-3,112/leu2 thr4 lys5 hml::ADE1/hml::ADE3 hmr::HYG ade3::GAL10::HO FS2::NAT/FS2</i>	(Deem et. al. 2008)
yWH271	AM1003	<i>MATα-KANMX-tel/MATα-inc ade1 met13 ura3 leu2-3,112/leu2 thr4 lys5 hml::ADE1/hml::ADE3 hmr::HYG ade3::GAL-HO FS2::NAT/FS2</i>	This study
NP667	yWH271	<i>rad59::LEU2MX</i>	This study
NP660	yWH271	<i>rad52-R70A</i>	This study
NP668	yWH271	<i>rad59::LEU2MX rad52-R70A</i>	This study

K. Resection analysis			
NP541	JKM139	<i>rad52-R70A</i>	This study
AP485	JKM139	<i>rad52::KANMX</i>	This study
NP729		<i>WT</i>	This study
NP814	NP729	<i>mre11::HPHMX</i>	This study
NP851	NP729	<i>yku70::NATMX</i>	This study
NP875	NP729	<i>yku70::NATMX mre11::HPHMX</i>	This study
L. Recombination assay on chromosome V			
yRA97		<i>hoΔ mat::hisG hmlΔ::hisG HMRA-stk ura3Δ851 trp1Δ63 leu2Δ::KAN hmrΔ::ADE3 ade3::GAL10::HO can1Δ::UR::HOcs::A3::TRP1 RA::LEU2</i>	(Anand et. al., 2014)
NP844	yRA97	<i>rad52-R70A</i>	This study
NP872	yRA97	<i>sir2::HPHMX</i>	This study

Appendix Table S3: Primers used in this study

Name	5' → 3' sequence	Comments
MATa distal Fw	ATTGGCTATACGGGACGG	To make <i>MAT</i> distal probe
MATa distal Rv	CTTAGCTTGTACCAGAGG	
Z probe Fw	CGCAACAGTATAATTTTATAAA	To make Z probe
Z probe Rv	ATGAGTGTATAAACAAACATTG	
SSA probe Fw	ATTGTACGCCAACTTAAGACC	To make SSA probe
SSA probe Rv	GATATCACCAAACATGTTGC	
ACT1 probe Fw	TCTTCCCATCTATCGTCCGGTAGAC	To make control <i>ACT1</i> probe
ACT1 probe Rv	GGTCAATACCGGCAGATTCC	
MATa resect Fw	CTAAAGTAGAGCAACATACATTACACA	MAT probe (resection)
MATa resect Rv	ACATCAAGATCGTTTATGGTTAAGATA	
BPL1 Fw	CCAGGATCAGTTAAGCATGCTG	To make control <i>BPL1</i> probe
BPL1 Rv	ATTCAGGGTGTGGTCCAGTGAG	
TRP1 probe Fw	GCATGGTATCACTACACACATC	To make <i>TRP1</i> probe
TRP1 probe Rv	AACCACACACTTTAACGAGCAT	
FEN2 probe Fw	CACCAATGCATATATATCCG	To make <i>FEN2</i> probe
FEN2 probe Rv	GAATAGTCGACCAGTCTAAC	
HML distal Fw	GGAAAATGCGTGTTCCCTTGT	To make HML distal probe
HML distal Rv	TCATTGCAAACGTCGGGCTTT	
H F1	gatatcttttaatggtgtctcactatcttccaataagactctaccagat TCGAG GTCGACGGTATC	To construct H-0 systems
H-0 R1	acttttcttttggagttttatacagtttccccgaaagaacaaaaatcaag CGCTCTAGAAGTAGTGGATC	
H-150 no gap R1	gtaatgtctctaaaagccataaatgagtttaatatcttgaagatt CGCTCTAGAAGTAGTGGATC	Use with H F1 to construct H-150 no-gap
kiTRP(III) F1	gggcttaacaggagccgccccatgcggaatcatgtctccgagcagaggact tttaatagag GGCCAGAAGACTAAGAGGTG	To make <i>kiTRP1</i> insertion cassette
kiTRP(III) R1	ttttcttttctcttttgcgagtagtaatacaccggaacggaaactaatg AAAGTGGAACGATCATTCACTATAT	
LEU2(III) F1	acaattcaagatctgtatcatttacactaacacaccccatttaatagag ATGTCTGCCCCTAAGAAGAT	To make <i>LEU2</i> insertion cassette
LEU2(III) R1	aatgatgcagtggcgatatcatatgcaaaagaaatgtaggaagtactcag TTAAGCAAGGATTTTCTTAACCTTCT	
KanMX(III) F1	acaattcaagatctgtatcatttacactaacacaccccatttaatagag GACATGGAGGCCAGAATAC	To make <i>KANMX</i> insertion cassette
KanMX(III) R1	aatgatgcagtggcgatatcatatgcaaaagaaatgtaggaagtactcag CAGTATAGCGACCAGCATTC	
gRNA-URA3(V)	GTTGATTATGACACCCGGTG	gRNA targeting <i>ura3-52</i>
<i>ura3</i> template	TCTTAACCCAACTGCACAGAACAAAA CCTGCAGGAAACGAAGATAAATCAAAA	Template to delete <i>URA3</i>

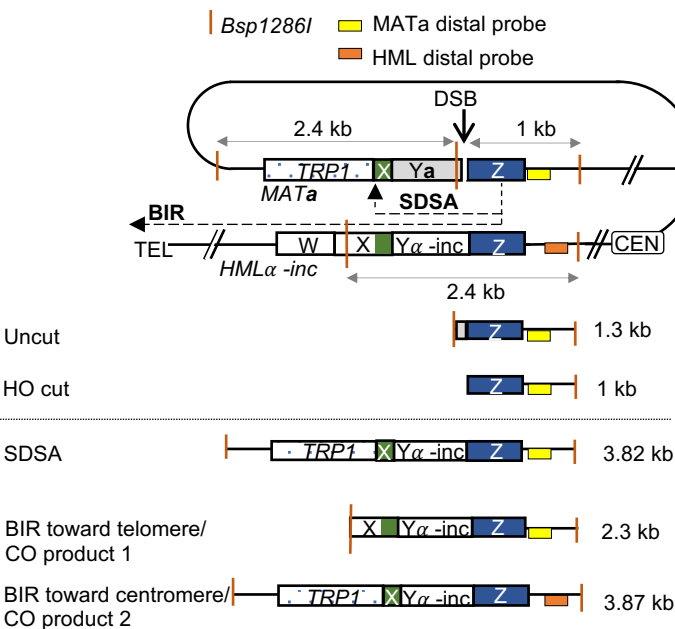
	CTGTATTATAAGTAAATGCATGTATACT AAACTCACAAATTAGAGC	
gRNA-RAD52	ACTCTCTTGGAGATATACTC	gRNA targeting <i>RAD52</i>
<i>rad52-R70A</i> template	CAAGAAATTAGGACCTGAGTATATCTC CAAGGCAGTTGGGTTTGAACAAGCA GGATTGCATACATCGAAGGT	Template to make <i>rad52-R70A</i>

*Primer sequences for other gene deletions are available upon request. All gene disruptions/alterations were confirmed by PCR. Point mutations were confirmed by Sanger sequencing.

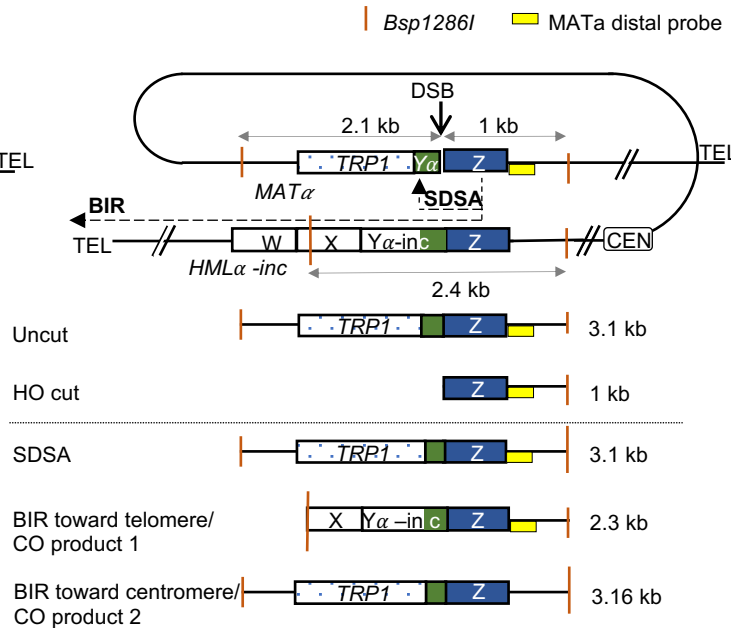
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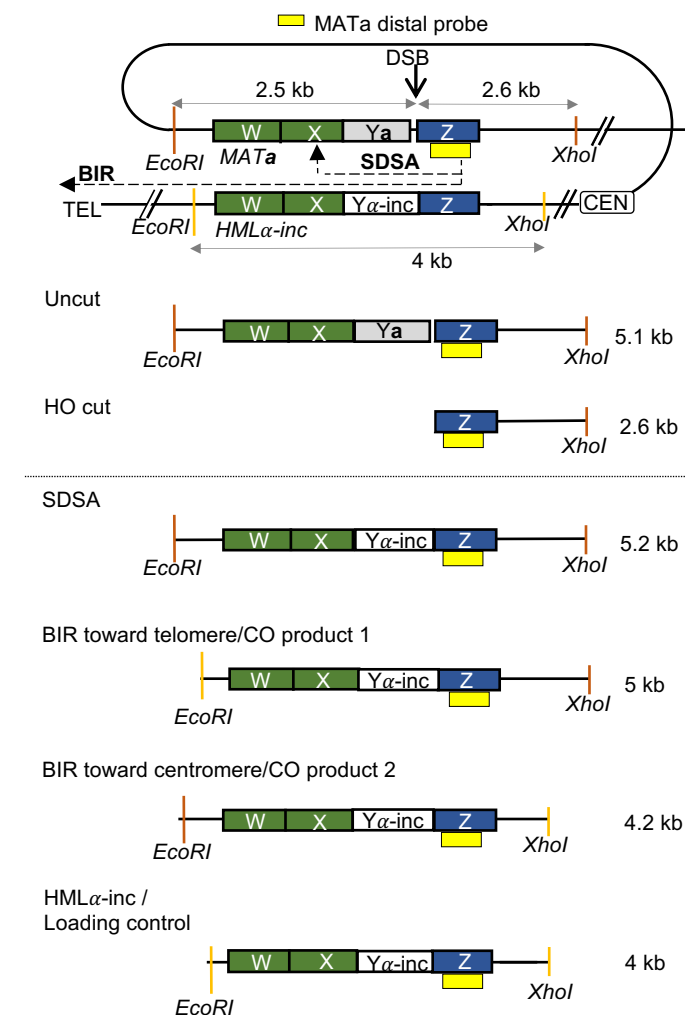
A. H-150



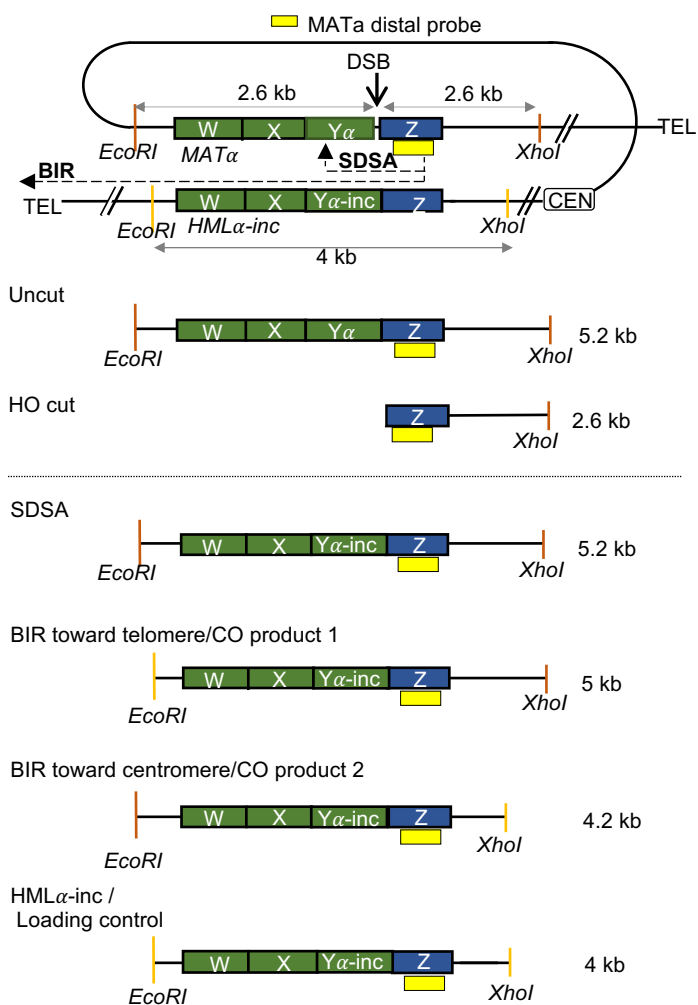
B. H-150 no-gap

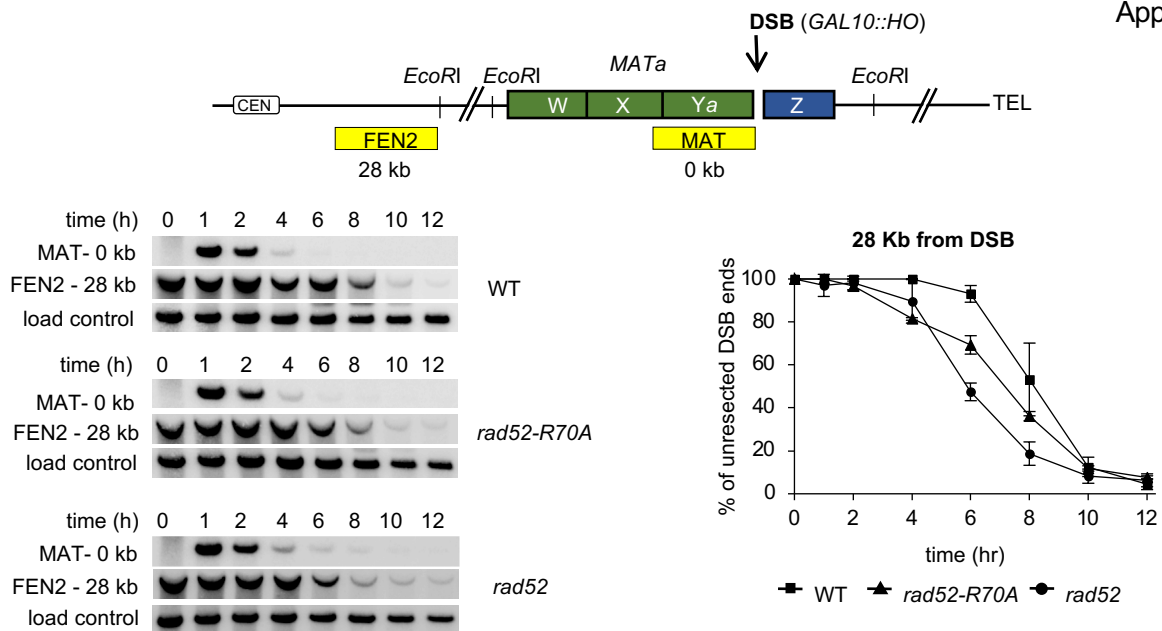
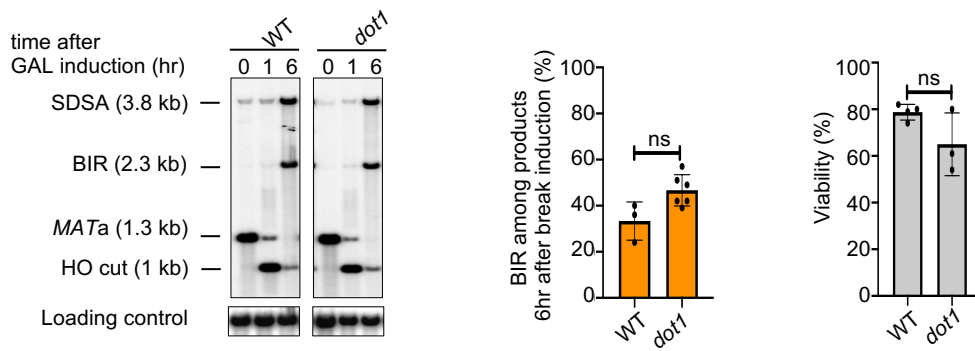
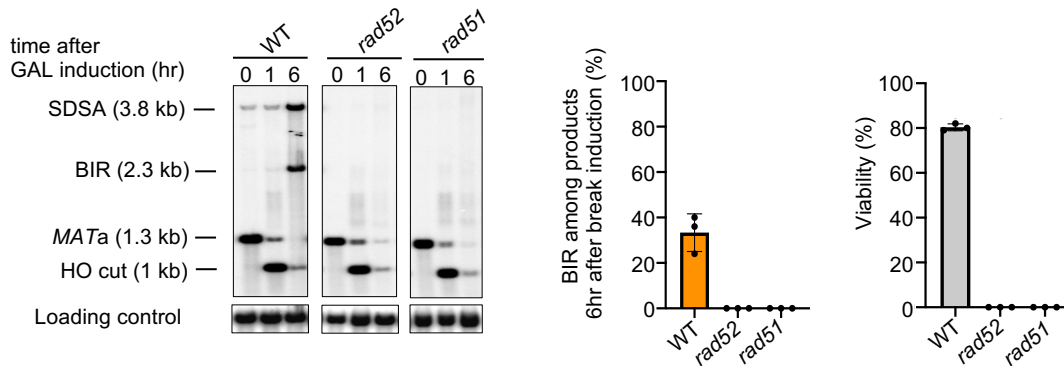


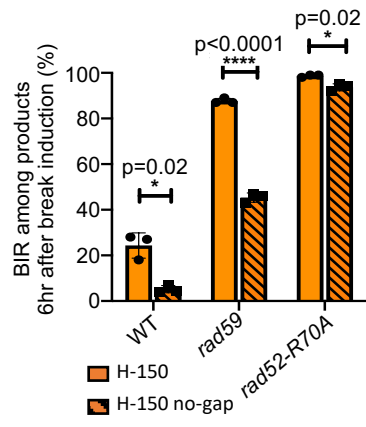
C. H-1400



D. H-2100



**B. H-150****C. H-150**



Appendix Figure S1. Schematic of recombination assays used in figure 1 and 2

Restriction enzyme sites, size of different repair outcomes, and the position of probes used for Southern blots are shown.

Appendix Figure S2. Role of resection, Rad52 and Rad51 in repair pathway choice

(A) Schematic of resection assay including sites of restriction enzyme *EcoRI* cleavage and location of *MAT* and *FEN2* probe (top), representative of Southern blots (bottom left) and graph showing kinetics of extensive resection measured 28 kb from DSB (bottom right) in WT, *rad52* Δ and *rad52-R70A* mutant cells.

(B) Representative Southern blots showing DSB repair products (left), percentage of BIR product among repair outcomes by 6 hr (middle) and viability (right) of WT and *dot1* Δ cells in H-150 assay.

(C) Representative Southern blots showing DSB repair products in WT, *rad52* Δ , and *rad51* Δ cells in H-150 assay (left), percentage of BIR products among repair outcomes by 6 hr (middle), and viability of indicated strains (right) are shown. The wild-type control is the same as in figure S2B as experiments in figures S2B and S2C are parallel.

Appendix Figure S3. Comparison of BIR product in H-150 assays with gap and without gap of WT, *rad59* Δ , *rad52-R70A*

The percentage of BIR products among repair outcomes by 6 hrs in H-150 and H-150 no-gap assays. The data were taken from the same experiments as shown in figure 1 and 2.