

**Table S1. Yeast strains**

Strain	Relevant genotype <sup>a</sup>	Comments/reference
SJR3714	<i>MATa lys2::IScel</i>	Derivative of MC42-2d Guo <i>et al.</i> 2017
SJR3939	<i>MATa lys2::IScel can1::lys2Δ3'-98%,IScelInc his3Δ::kanMX-pGAL-IScel</i>	Guo <i>et al.</i> 2017
SJR4015	<i>MATa lys2::CORE-UK</i>	CORE-UK cassette inserted into the <i>LYS2</i> locus of SJR3714
SJR4016	<i>MATa can1::lys2Δ3'-98%,IScelInc</i>	Donor only Spore from HLK1042-2c x SJR3939
SJR4031	<i>MATa can1::lys2Δ3'-98%,IScelInc-URA3-hisG</i> (= 98% donor/ <i>hisG</i> )	SJR4016 transformed with <i>URA3-hisG</i> fragment amplified from pNKY51
SJR4194	<i>MATa lys2,6Amut::IScel</i>	CORE-UK of SJR4015 replaced with mutated 6A run
SJR4195	<i>MATa hisG-lys2,6Amut::IScel-URA3-hisG</i> (= <i>hisG</i> /recipient)	SJR4194 transformed with <i>hisG-URA3-hisG</i> fragment (pNKY51) and subsequently for <i>URA3</i> loss
SJR4196	<i>MATa hisG</i> /recipient + 98% donor/ <i>hisG</i>	Spore from SJR4195 x SJR4031
SJR4258	<i>MATa hisG</i> /recipient + 98% donor/ <i>hisG</i> + <i>his3Δ::hph-pGAL-IScel</i> (= I- <i>Scel</i> )	SJR4196 transformed with <i>his3Δ::hph-pGAL-IScel</i> from pSR952
SJR4535	<i>MATa hisG</i> /recipient + 98% donor/ <i>hisG</i> + I- <i>Scel mlh1Δ::loxP-TRP1-loxP</i>	SJR4258 transformed with <i>mlh1Δ::loxP-TRP1-loxP</i> fragment
SJR4691	<i>MATa hisG</i> /recipient + 98% donor/ <i>hisG</i> + I- <i>Scel msh6Δ::loxP-kanMX-loxP</i>	SJR4258 transformed with <i>msh6Δ::loxP-kanMX-loxP</i> fragment
SJR4800	<i>MATa hisG</i> /recipient + 98% donor/ <i>hisG</i> + I- <i>Scel msh6Δ::loxP-kanMX-loxP rad14Δ::loxP-TRP1-loxP</i>	SJR4691 transformed with <i>rad14Δ::loxP-TRP1-loxP</i> fragment
SJR4982	<i>MATa HOM3</i> recipient + 98% donor + galactose-inducible I- <i>Scel mlh1Δ::loxP-TRP1-loxP msh6Δ::loxP-KanMX-loxP</i>	SJR4535 transformed with <i>msh6Δ::loxP-KanMX-loxP</i> fragment
SJR5000	<i>MATa hisG</i> /recipient <i>can1::lys2Δ3'-100%,I-ScelInc</i> (= 100% donor)	SJR4195 transformed with pSR1154 fragment to introduce 100%-identical donor at <i>CAN1</i>
SJR5003	<i>MATa hisG</i> /recipient 100% donor/ <i>hisG</i>	SJR5000 transformed with <i>URA3-hisG</i> fragment from pNKY51
SJR5007	<i>MATa hisG</i> /recipient 100% donor/ <i>hisG mlh1Δ::loxP-TRP1-loxP</i>	Spore from SJR5003 x SJR4535
SJR5036	<i>MATa hisG</i> /recipient + 100% donor/ <i>hisG</i> + I- <i>Scel</i>	Spore from SJR5007 x SJR4691
SJR5037	<i>MATa hisG</i> /recipient + 100% donor/ <i>hisG</i> + I- <i>Scel mlh1Δ::loxP-TRP1-loxP</i>	Spore from SJR5007 x SJR4691
SJR5038	<i>MATa hisG</i> /recipient + 100% donor/ <i>hisG</i> + I- <i>Scel msh6Δ::loxP-KanMX-loxP</i>	Spore from SJR5007 x SJR4691
SJR5039	<i>MATa hisG</i> /recipient + 100% donor/ <i>hisG</i> + I- <i>Scel mlh1Δ::loxP-TRP1-loxP msh6Δ::loxP-KanMX-loxP</i>	Spore from SJR5007 x SJR4691

<sup>a</sup> All strains were derived from W303 derivatives MC42-2d (*MATa RAD5 leu2-3,112 his3-11,15 ura3-1 trp1-1 ade2-1*) and HLK1042-1c (*MATa RAD5 leu2-3,112 his3-11,15 ura3-1 trp1-1 ade2-1 hom3-10*). Only the changes introduced by transformation or mating are given. With the exception of HLK1042-1c, all strains are *HOM3*.

**Table S2. Observed and corrected numbers of NCO and CO events**

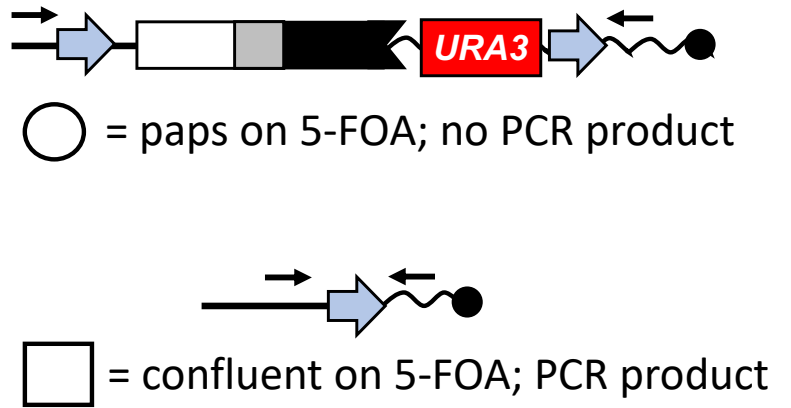
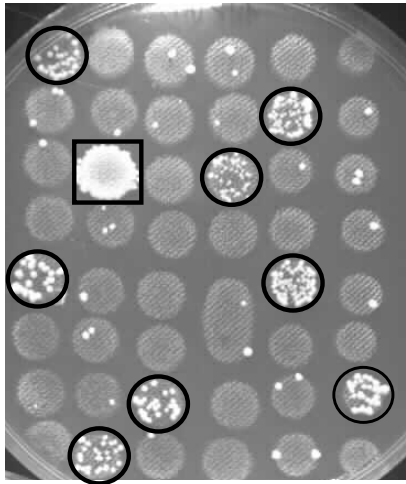
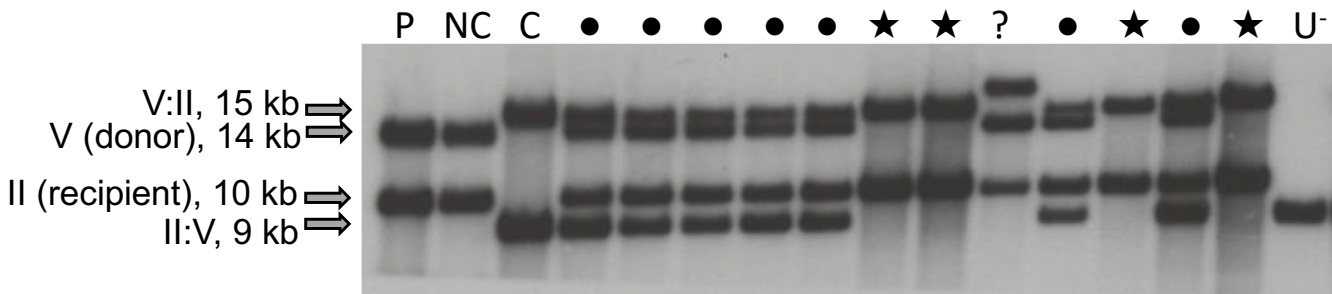
Substrates	Strain	Observed numbers of events					Corrected numbers of events				
		NCO	CO	CO+NCO	Ura-	Total	NCO <sup>1</sup>		CO <sup>2</sup>		Total
							No.	Prop.	No.	Prop.	
100%	WT (SJR5036)	228	23	36	0	287	264	0.763	82	0.237	346
	<i>mlh1</i> Δ (SJR5037)	230	15	39	0	284	269	0.796	69	0.204	338
	<i>msh6</i> Δ (SJR5038)	235	14	40	0	289	275	0.802	68	0.198	343
	<i>msh6</i> Δ <i>mlh1</i> Δ (SJR5039)	232	18	35	0	285	267	0.790	71	0.210	338
98%	WT (SJR4258)	422	29	14	13	478	436	0.816	98	0.184	534
	<i>mlh1</i> Δ (SJR4535)	438	18	14	1	471	452	0.897	52	0.103	504
	<i>msh6</i> Δ (SJR4691)	388	44	41	4	477	429	0.758	137	0.242	566
	<i>msh6</i> Δ <i>mlh1</i> Δ (SJR4982)	215	30	41	0	286	256	0.717	101	0.283	357

<sup>1</sup> Corrected NCO = observed(NCO) + observed(CO+NCO)

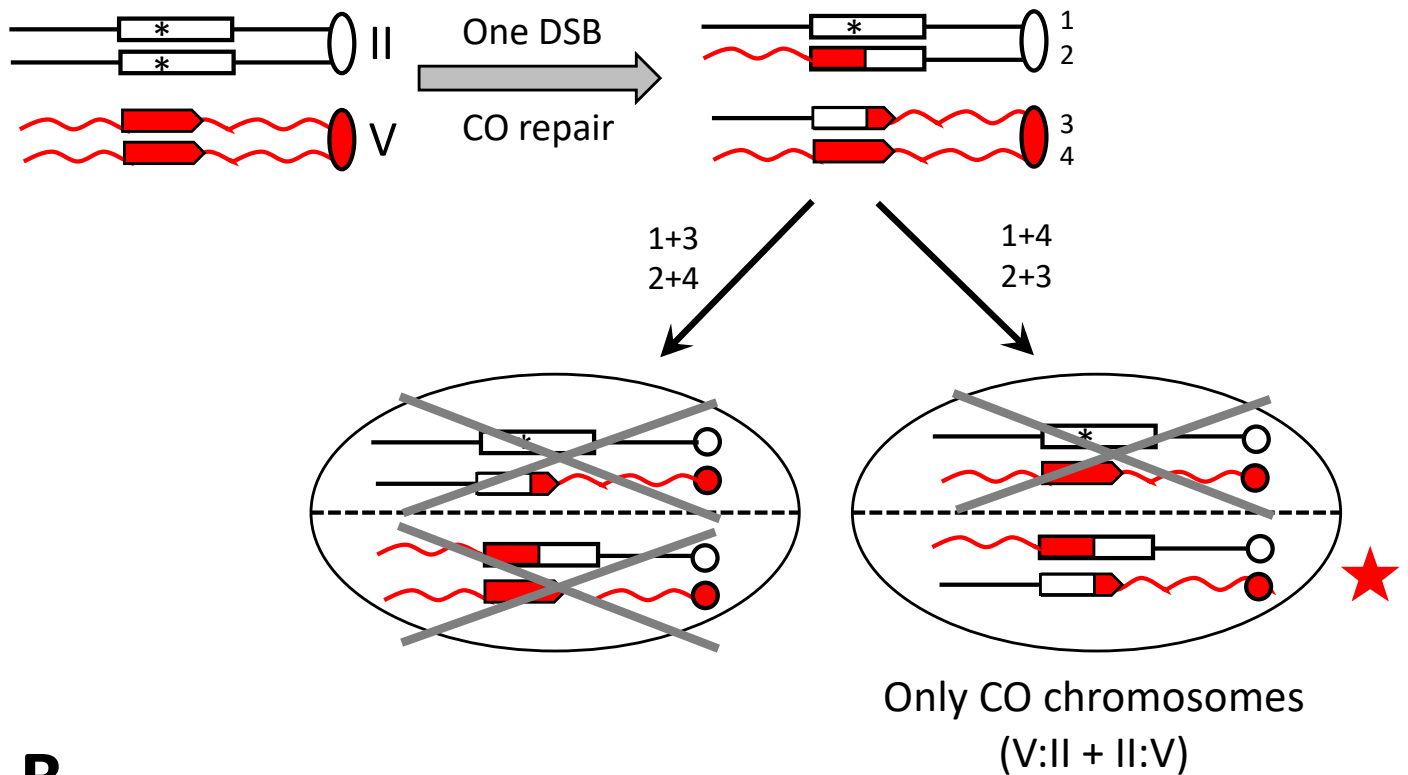
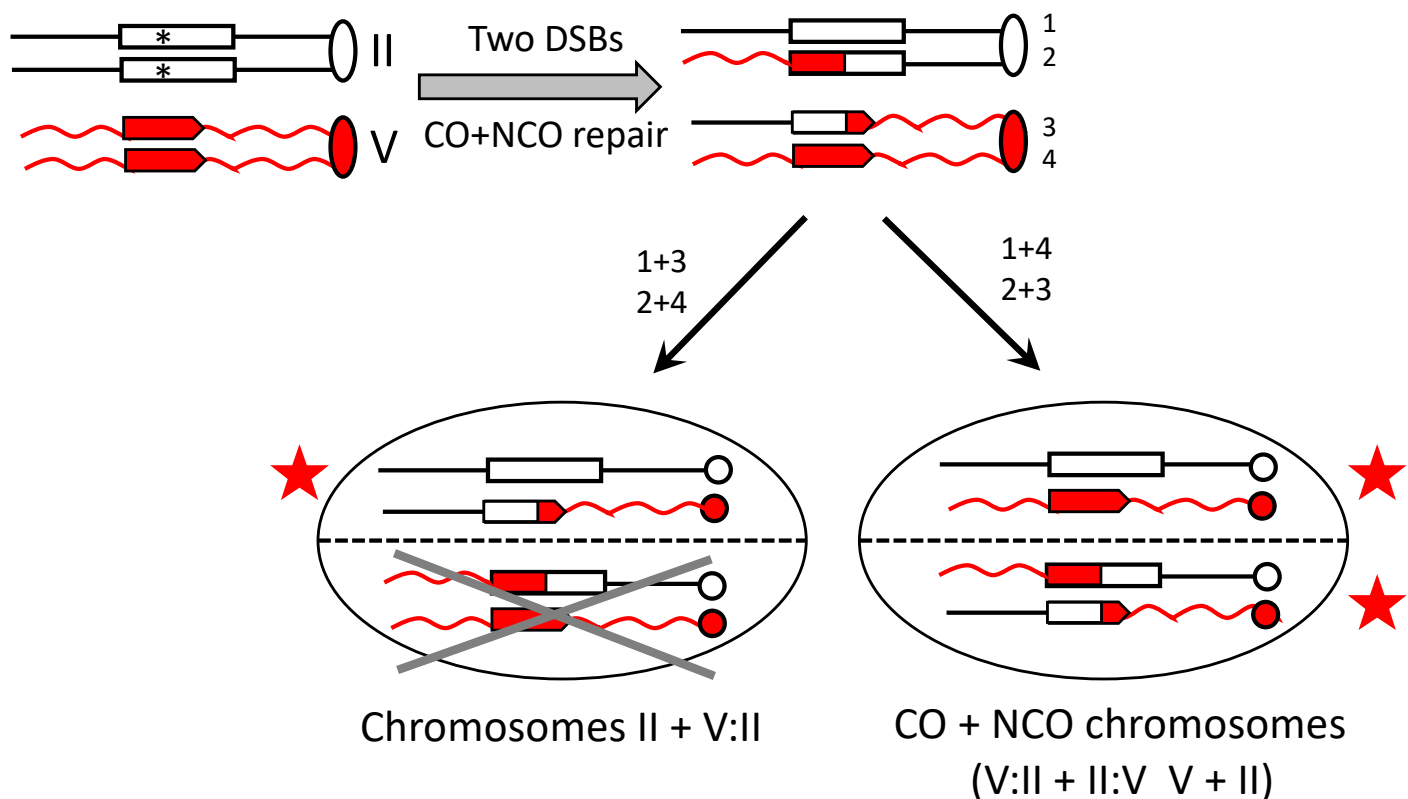
<sup>2</sup> Corrected CO = 2(observed CO + Ura<sup>-</sup>) + observed(CO+NCO)

GCGCTTTGAAAAGGCCAAACTATCGAAGGACAAGCCAACCATAATCAAGGTAACACTACTACAATTGGATTGGGTCCCTACAACAGGGTACTGCTGGTGTTCATGGTCCGCTTTGAAGGCAGATGATGTTAAACAGTTGAAGAAGAGGTGGGGTTTGC  
 ACCCAAATAAATCATTTGTAGTACCTCAAGAGGTGTAACGATTATTATAAGAAGACTGTTGTGGAACCCGGTCAAAAACCTAATGAGGAATGGGATAGGATGTTTGAAGAATACAAAACCAAATTTCCCGAGAAGGTTAAAGAATTGCAAAGAAGATTGA  
 ATGGTGAAGTACCGGAAGGTTGGGAAAAGCATTACCAGAACTTACTCCGGACGACGATGCTCTGGCAACAAGAAAGACATCCCAGCAGGTGCTGACGAACATGGTCCAAGTTTTGCCTGAATTGATCGTTGGTCTGCCGATTGACACCTTCGAATC  
 TGACAAGGTGGGAAGGCGCGGTAGATTTCACACTCCATTACCCAACTAGGTAACATATGCAGGAAGGTACATTAGATACGGTGTGAGGGAACACGGAATGGGTGCCATTATGAACCGGTATCTCTGCCTTTGGTGCAAACTACAAGCCTTACGGTGGTA  
 CCTTTTGAACCTCGTCTCTTATGCTCAGGAGCCGTTAGGTTAGCCGCTTGTCTGGTAAACGAGTCATTTGGGTTGCAACACATGACTCTATCGGGCTTGGTGGAGATGGTCAACGCACCAACCTATTGAAACTCTGGCTCACTTGAGGGCTATTTC  
 CAAACAATGCATGTATGGAGACCTGCTGATGTTAACGAACTTCTGCTGCTATTATTCTGCTATCAAATCTGGTGAACACCATCTGTTGTGGCTTTATCAGACAGAATCTTCTCAATTGGAGCATTCTCTTTTAAAAAGCCTTGAAGGGTGGCT  
 ATGTGATCCATGACGTGGAGAATCCTGATATTATCCTGGTGTCAACAGGATCAGAAGTCTCCATTCTATAGATGCAGCCAAAAAATTGTACGATACTAAAAAATCAAAGCAAGAGTTGTTCCCTGCCAGACTTTTATACTTTTGACAGGCAAAGTG  
 AAGAATACAGATTCTCTGTTTACCAGACGGTGTCCGATCATGTCTTTGAAGTATTGGCTACTTCAAGCTGGGTAAGTATGCTCATCAATCGTTCGGACTCGACGAATTTGGTCTGTTGAGGCAAGGGGCTGAAATTTACAAATTTGTCGATTTCA  
 CAGCGGACGGTGTTCGCTCAAGGGCTGAAAAGACAATCAATTAATAAGGAAAGCAGTTGCTTTCTCTATGGGAAGAGCTTTCTAAGTCTGAAGAAGTAAACAGTTCTTTGCTATTTCACACTTCTGGTTGATGGTCACTTGTCTGCCTGAAATAT  
 ATATATATGTATGACATATGACTTGTGTTTTCTTTTTGTGCCCTTGTACGCTCTATATTCAATGAAACTGATTATTCGATTTTCTTCTGCTGACCGCTTTAGAGGCATCGCACAGTTTTAGCGAGGAAAACCTTCAATAGTTTTGCCAGCGGAATT  
 CCACCTTGCATTAACATAAAAAATTCGGCGGTTTTTCGCGTGTGACTCAATGTCGAAATACCTGCCTAATGAACATGAACATCGCCAAATGTATTGAAGACCCGCTGGGAGAAGTTCAAGATATATAAGTAACAAGCAGCCAATAGTATAAAAAAA  
 ATGTGAGTTTATTACCTTTCTGGAATTCAGTAAAAACTGCTAATTATAGAGAGATATCACAGAGTTACTACTAATGACTAAAGAAAAGTCTGGATAGAGAAGTTGGATAATCCAACCTTTTCAGTGTACCACATGACTTTTTACGCCACAAC  
 AAGAACCTTATACGAAACAAGTACATATTGTTACAGCTACCTCAGCTCGATGTCCTCATGATAGTTTTCTAACAAATACGCTGTGCTTTGAGTGTATGGGCTGCATTGATATAATAGATAACCGGTGACGATGATATGTTCTTTATATTGCGA  
 ATAACAAAATCTTAAAGATTCAATATCAACCAACGTGGTCATTTAATGAGCTGTATTCTACAATTAACAATGAGTTGAACAAGCTCAATCTATTGAGGCCAATTTTTCTCTTTCGACGAGCTAGCTGAATCAATCAAAGTTGCCAAGATCAATTACCTT  
GTTATCCCTAGATCTGGAAGGACCCCTCAGTTGTTCCGTTTGGCCTTTTGGAAAACCAAGATTTCAAATTAGACGAGTTCAAGCATCATTTAGTGGACTTTGCTTTGAATTTGGATACCAGTAATAATGCGCATGTTTTGAACTTAATTTATAATTC  
 AGCTTACTGTATTGAAAGAAAGATTAACCAATTTGTTGGGACCAATTTACTCAATATTTGACTGCTGCGCTAAGCGATCCATCCAATTCATAACTAAAATCTCTCTGATCACCGCATCATCCAAGGATAGTTTACCTGATCCAACCTAAGAACTTGGGC  
 TGGTGCATTTCTGTTGGGTGTATTCCAGACATTTCCAGGACAATGCTGAAGCCTTCCCAGAGAGAACCCTGTGTTGTGGAGACTCCAACTAAATTCGACAAAGTCCCGTCTTTCACTTATCGCGACATCAACCGCACTTCTAACATAGTTGCCCAT  
 TATTTGATTAACACAGGTATCAAAGAGGTGATGTAGTATGATCTATTCTTCTAGGGGTGTGATTGATGGTATGTGTGATGGGTGCTTTGAAGCCGGCGCAACCTTTTCAGTTATCGACCCCTGCATATCCCCAGCCAGACAAACATTTACTTA  
 GGTGTTGCTAAACACAGTGGGTGATGTTATTAGAGCTGCTGGACAATTTGGATCAACTAGTAGAAGATTACATCAATGATGAATTTGGAGATTGTTTCAAGAATCAATCCATCGCTATTCAGAAAATGGTACCATTGAAGGTGGCAAATTTGGACAAT  
 GGCGAGGATGTTTTGGCCCATATGATCACTACAAAGACACCAGAACAGGTGTTGTAGTTGGACCCGATTTCAACCCCAACCCATCTTTTACATCTGGTTCGGAAGTATTCCTAAGGTGTTCTTGGTAGACATTTTTCTTGGCTATTATTTCAAT  
 TGGATGTCAAAAGGTTCAACTTAACAGAAAATGATAAATTCACAATGCTGAGCGGTATTGCACATGATCCAATTCAAAGAGATATGTTTACACCATTATTTTTAGGTGCCCAATTGTATGTCCCTACTCAAGATGATATTGGTACACCGGGCCGTTTA  
 GCGGAATGATGAGTAAGTATGGTGCACAGTTACCCATTTAACACCTGCTATGGGTCAATTACTTACTGCCAAGCTACTACACCATTCCCTAAGTTACATCATGCGTCTTTGTGGGTGACATTTTAAACAAAACGTGATTGTCTGAGTTTACAACC  
 TTGGCAGAAAATTGCCGTATTGTTAATATGTACGGTACCCTGAAACACAGCGTGCAGTTTCTTATTTCGAAGTTAAATCAAAAAATGACGATCCAACCTTTTTGAAAAAATTGAAAGATGTCATGCTGCTGGTAAAGGTATGTTGAACGTTACGCTA  
 CTAGTTGTTAACAGGAACGATGCTACTCAAATATGTGGTATTGGCGAAAATAGGTGAGATTTATGTTCTGTCAGGTGGTTTGGCCGAAGGTTATAGAGGATTACCAGAATTGAATAAAGAAAAATTTGTGAACAACCTGGTTTGTGAAAAAGATCACTGG  
 AATTATTTGGATAAGGATAATGGTGAACCTTGGAGACAATTTGGTTAGGTCCAAGAGATAGATTGTACAGAACGGGTGATTTAGGTGCTTATCTACAAACCGGTGACTGTAATGTTGCGGTAGGGCTGATGATCAAGTTAAAAATTCGTGGGTTCAGA  
 ATCGAATTAGGAGAAATGATACGCACATTTCCCAACATCCATTGGTAAGAGAAAACATTAATTTGTTTCGCAAAAATGCCGACAATGAGCCAACATTGATCACATTTATGGTCCCAAGATTTGACAAGCCAGATGACTTGTCTAAGTTCCAAAGTGAT  
 GTTCCAAAGGAGGTTGAAACTGACCCATAGTTAAGGGCTTAATCGGTTACCATCTTTTATCCAAGGACATCAGGACTTTCTTAAAGAAAAGAT

**Figure S1.** Homology between *lys2* substrates. The sequence shown is that of the recipient allele. The ATG start codon is red; the inserted I-SceI cut site is highlighted yellow and the region flanked by nicks is underlined. The SNPs that are present in the donor allele are above the sequence and are highlighted gray.

**A****B**

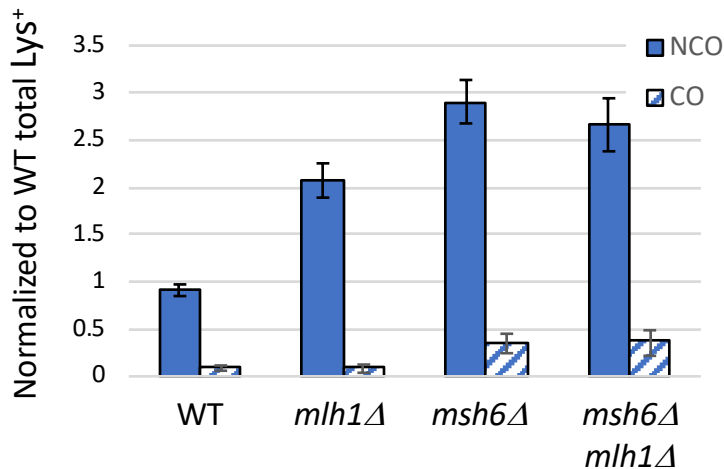
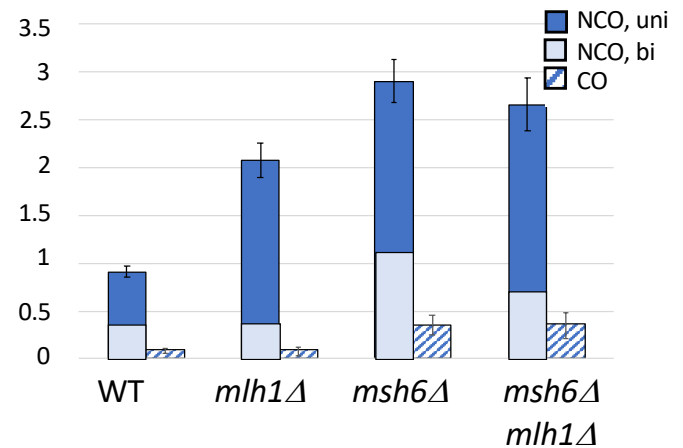
**Figure S2.** Characterization of CO and NCO events. **(A)** Phenotype screen for CO events on 5-FOA medium. CO events (circles) pap at a high frequency because of *hisG* repeats that flank the *URA3* marker while NCO events produce few paps on 5-FOA. A secondary CO between the *hisG* repeats results in confluent growth (box). Only fully Ura<sup>-</sup> COs produce a PCR product with the indicated primers (small horizontal arrows). **(B)** Southern analysis of colonies that contain both a CO and NCO chromosome by PCR. Lanes marked with a dot contain both CO and both normal chromosomes; lanes marked with a star contain only chromosomes V:II and II. P, parent; NC, NCO; C, CO; U<sup>-</sup>, Ura<sup>-</sup> colony.

**A****B**

**Figure S3.** Chromosome segregation and cell viability following DSB repair. **(A)** Only one sister chromatid is cleaved by I-SceI and repair generates reciprocal CO products. PCR only detects a CO chromosome. **(B)** Both sister chromatids are cleaved; one is repaired as a CO and the other as a NCO. PCR detects both a CO and NCO chromosome and a Southern blot detects either chromosomes II + II:V or all four chromosomes. Gray X, Lys<sup>-</sup> or inviable; red star, Lys<sup>+</sup> and viable.

**A****Uncorrected numbers and proportions of COs and NCOs produced from a single DSB**

Substrates	Strain	Observed numbers of events				Uncorrected total events			
		NCO	CO	Ura-	Total	NCO		CO	
						No.	Prop.	No.	Prop.
100%	WT (SJR5036)	228	23	0	251	228	0.908	23	0.092
	<i>mlh1Δ</i> (SJR5037)	230	15	0	245	230	0.939	15	0.061
	<i>msh6Δ</i> (SJR5038)	235	14	0	249	235	0.944	14	0.056
	<i>msh6Δ mlh1Δ</i> (SJR5039)	232	18	0	250	232	0.928	18	0.072
98%	WT (SJR4258)	422	29	13	464	422	0.909	42	0.091
	<i>mlh1Δ</i> (SJR4535)	438	18	1	457	438	0.958	19	0.042
	<i>msh6Δ</i> (SJR4691)	388	44	4	436	388	0.890	48	0.110
	<i>msh6Δ mlh1Δ</i> (SJR4982)	215	30	0	245	215	0.878	30	0.122

**B****C**

**Figure S4.** Uncorrected numbers, proportions and frequencies of COs and NCOs. **(A)** Only colonies that resulted from repair of a single DSB were considered; the CO+NCO class from Table S1 was omitted. The total COs correspond to the sum of the CO and Ura<sup>-</sup> classes without the additional correction for loss of COs due to inviability. **(B)** The NCO and CO frequencies for 98%-identical substrates were calculated as in Figure 3B, but the uncorrected proportions in (A) were used. **(C)** Uncorrected version of Figure 5, which was calculated using uncorrected proportions in (A).