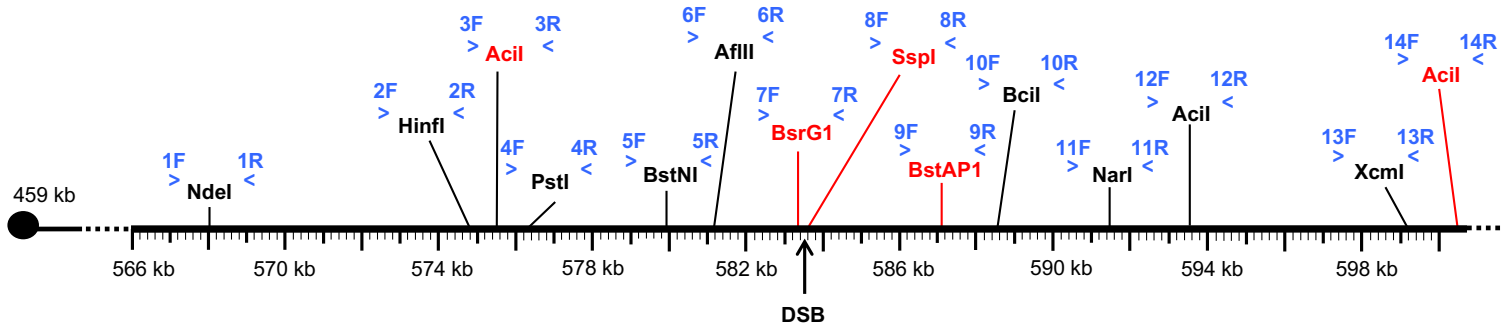


Figure S1. **Primers pairs and restriction sites used to diagnose genetic linkages in spores.** The right arm of chromosome IV is shown, with SGD coordinates indicated. Primer pairs are numbered and are in blue. The W303- or YJM-specific restriction site within each corresponding PCR fragment is in red or black, respectively. The position of the initiating DSB is indicated.

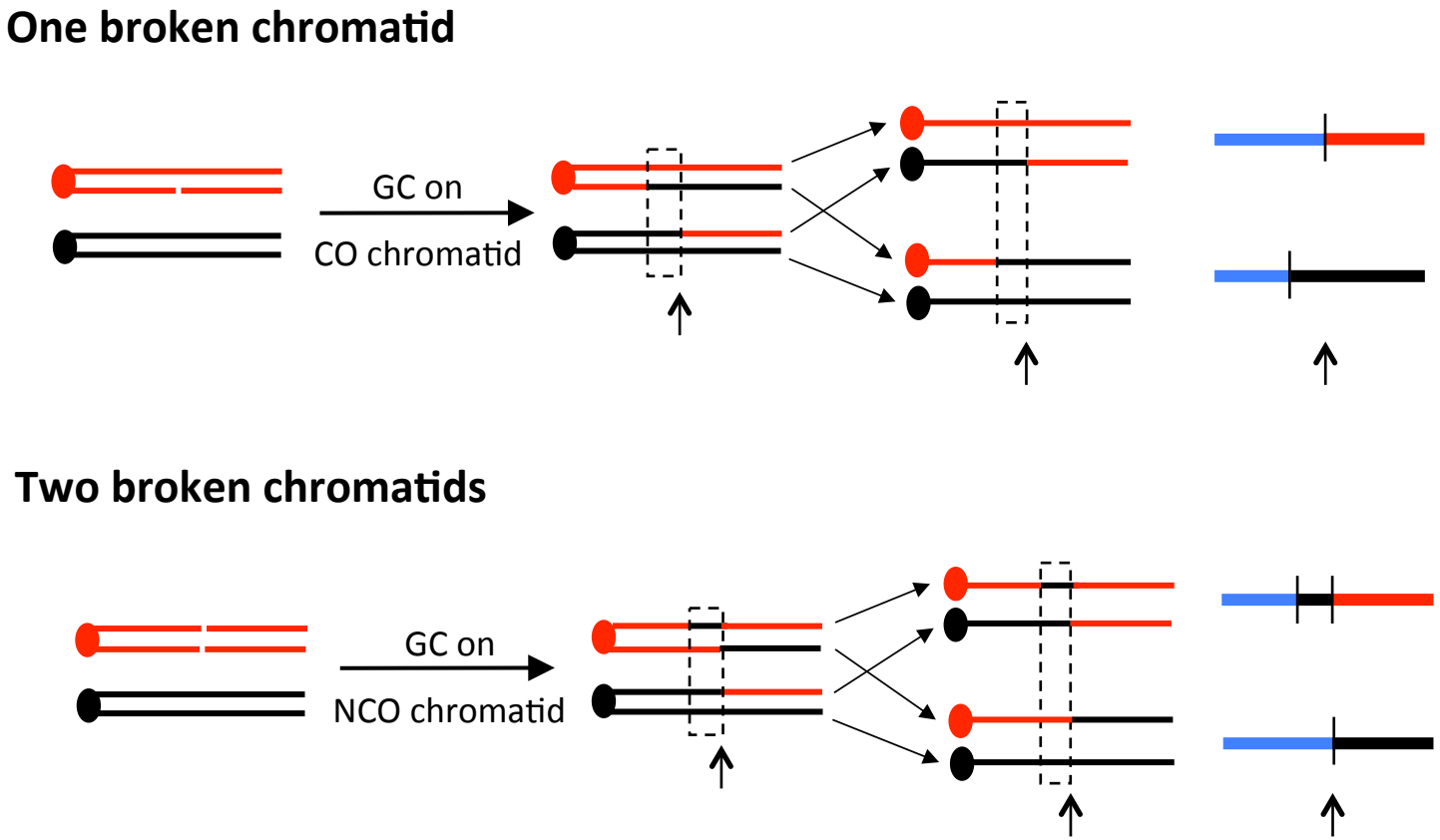
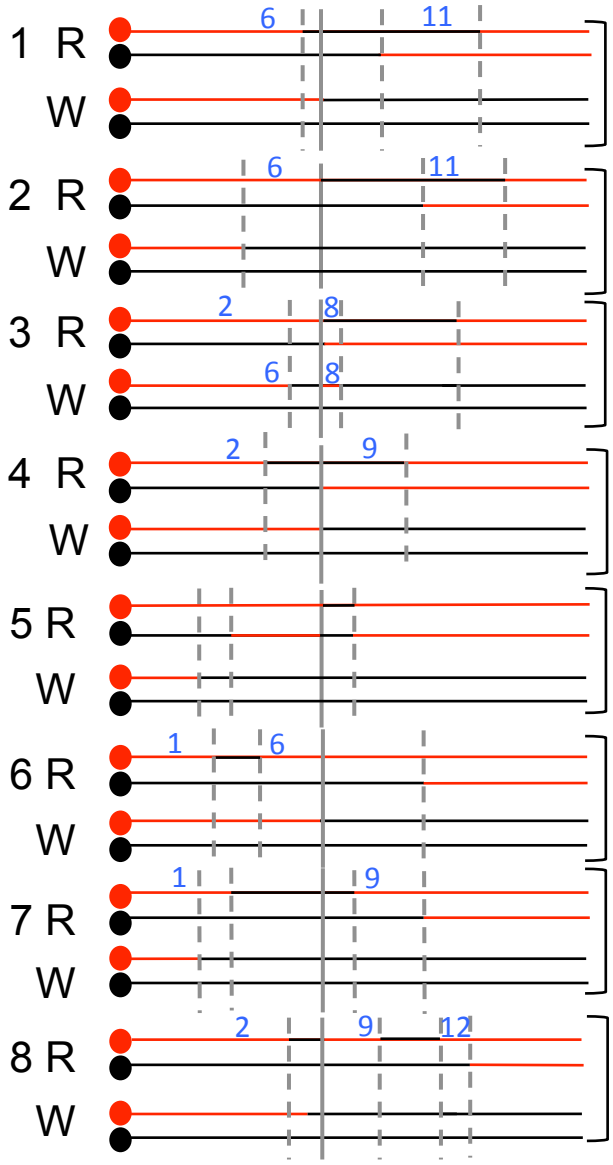
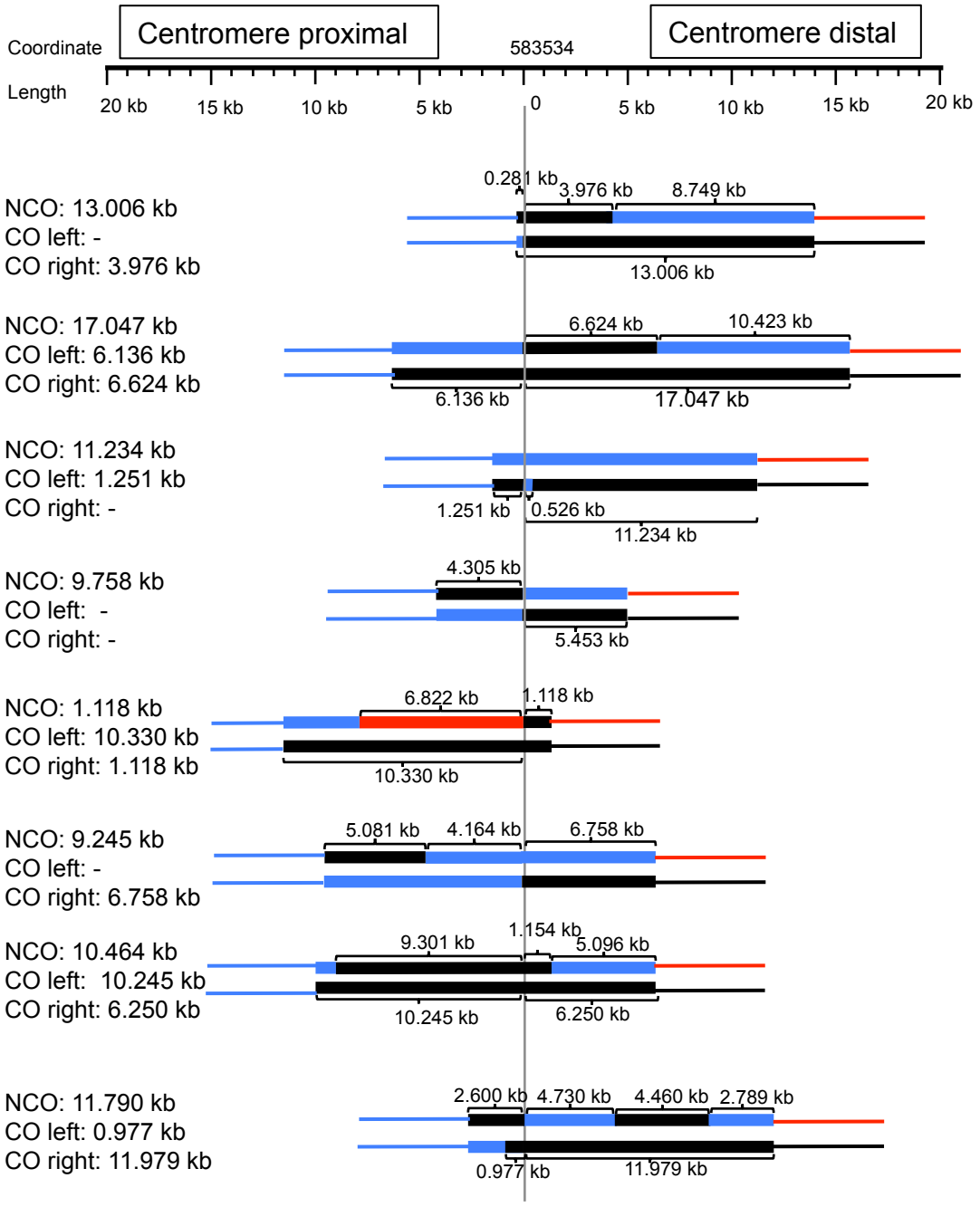
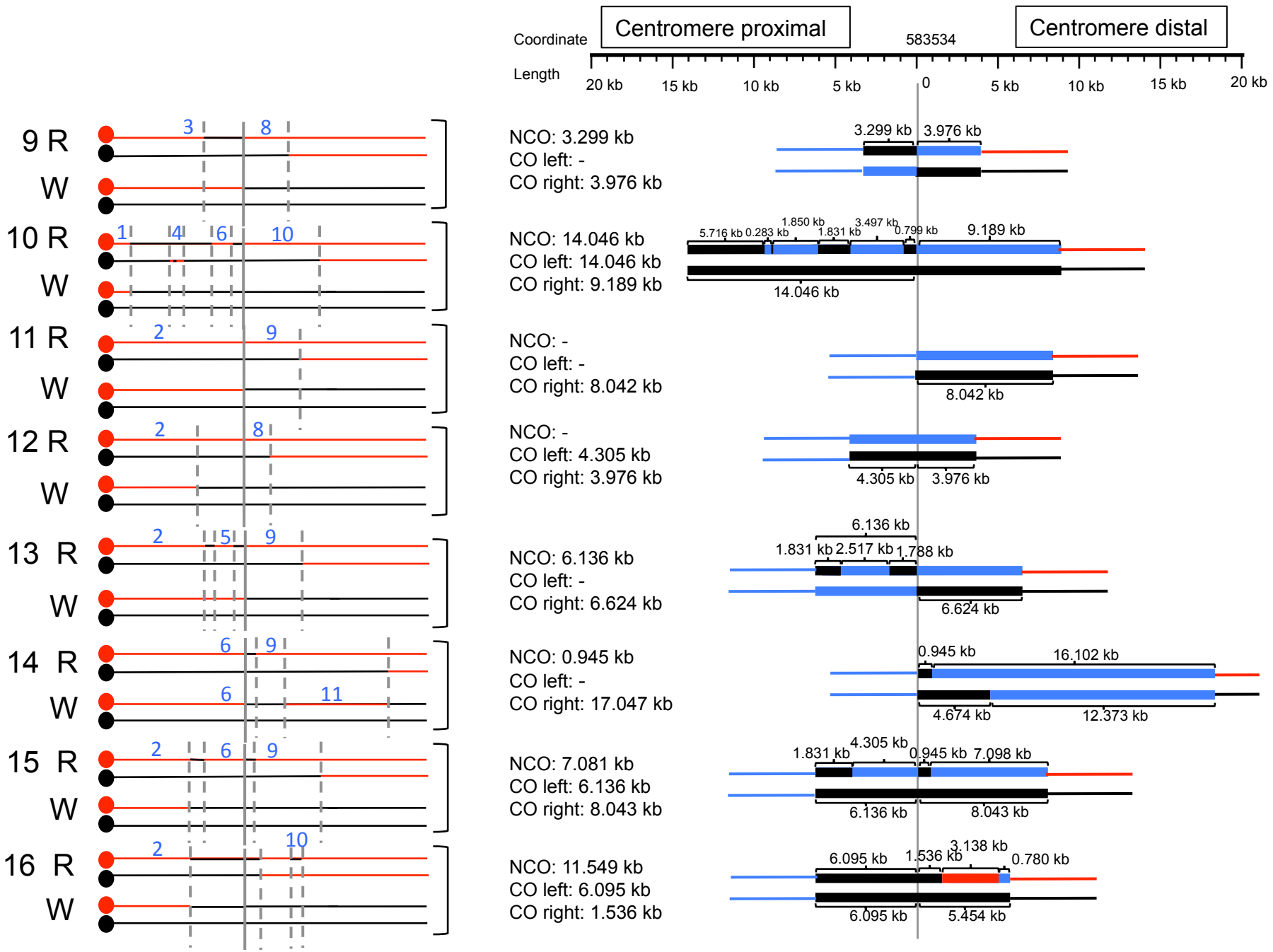


Figure S2. GC pattern when there are one versus two broken chromatids. Red and black lines correspond to W303- and YJM-specific chromosomal segments, respectively; red and black circles represent W303 and YJM centromeres, respectively. Vertical arrows indicate the DSB positions. When only one chromatid is broken (top panel) the GC tract is always located on the CO chromatid with the W303 centromere. In the abbreviated red/black/blue depiction (blue indicates heterozygosity), there is a single transition between heterozygosity and homozygosity in each sector (blue to red and blue to black in the red and white sectors, respectively). When both chromatids are broken and repaired, however, the GC tract can be on the NCO, W303 chromatid instead of on the CO chromatid (lower panel). The segregation of the GC-containing, NCO chromatid with a CO chromatid leads to two transitions (blue to black to red) in the red sector.


1 R
W
6
11
2 R
W
6
11
3 R
W
2
8
6
8
4 R
W
2
9
5 R
W
6 R
W
1
6
7 R
W
1
9
8 R
W
2
9
12


Coordinate
Length
Centromere proximal
583534
Centromere distal
20 kb
15 kb
10 kb
5 kb
0
5 kb
10 kb
15 kb
20 kb
NCO: 13.006 kb
CO left: -
CO right: 3.976 kb
0.281 kb
3.976 kb
8.749 kb
13.006 kb
NCO: 17.047 kb
CO left: 6.136 kb
CO right: 6.624 kb
6.624 kb
10.423 kb
6.136 kb
17.047 kb
NCO: 11.234 kb
CO left: 1.251 kb
CO right: -
1.251 kb
0.526 kb
11.234 kb
NCO: 9.758 kb
CO left: -
CO right: -
4.305 kb
5.453 kb
NCO: 1.118 kb
CO left: 10.330 kb
CO right: 1.118 kb
6.822 kb
1.118 kb
10.330 kb
NCO: 9.245 kb
CO left: -
CO right: 6.758 kb
5.081 kb
4.164 kb
6.758 kb
NCO: 10.464 kb
CO left: 10.245 kb
CO right: 6.250 kb
9.301 kb
1.154 kb
5.096 kb
10.245 kb
6.250 kb
NCO: 11.790 kb
CO left: 0.977 kb
CO right: 11.979 kb
2.600 kb
4.730 kb
4.460 kb
2.789 kb
0.977 kb
11.979 kb


Coordinate
Centromere proximal
583534
Centromere distal
Length
20 kb
15 kb
10 kb
5 kb
0
5 kb
10 kb
15 kb
20 kb
9 R
W
3
8
NCO: 3.299 kb
CO left: -
CO right: 3.976 kb
3.299 kb
3.976 kb
10 R
W
1
4
6
10
NCO: 14.046 kb
CO left: 14.046 kb
CO right: 9.189 kb
5.716 kb
0.283 kb
1.850 kb
1.831 kb
3.497 kb
0.799 kb
9.189 kb
14.046 kb
11 R
W
2
9
NCO: -
CO left: -
CO right: 8.042 kb
8.042 kb
12 R
W
2
8
NCO: -
CO left: 4.305 kb
CO right: 3.976 kb
4.305 kb
3.976 kb
13 R
W
2
5
9
NCO: 6.136 kb
CO left: -
CO right: 6.624 kb
6.136 kb
1.831 kb
2.517 kb
1.788 kb
6.624 kb
14 R
W
6
9
6
11
NCO: 0.945 kb
CO left: -
CO right: 17.047 kb
0.945 kb
16.102 kb
4.674 kb
12.373 kb
15 R
W
2
6
9
NCO: 7.081 kb
CO left: 6.136 kb
CO right: 8.043 kb
4.305 kb
1.831 kb
0.945 kb
7.098 kb
6.136 kb
8.043 kb
16 R
W
2
10
NCO: 11.549 kb
CO left: 6.095 kb
CO right: 1.536 kb
3.138 kb
6.095 kb
1.536 kb
0.780 kb
6.095 kb
5.454 kb

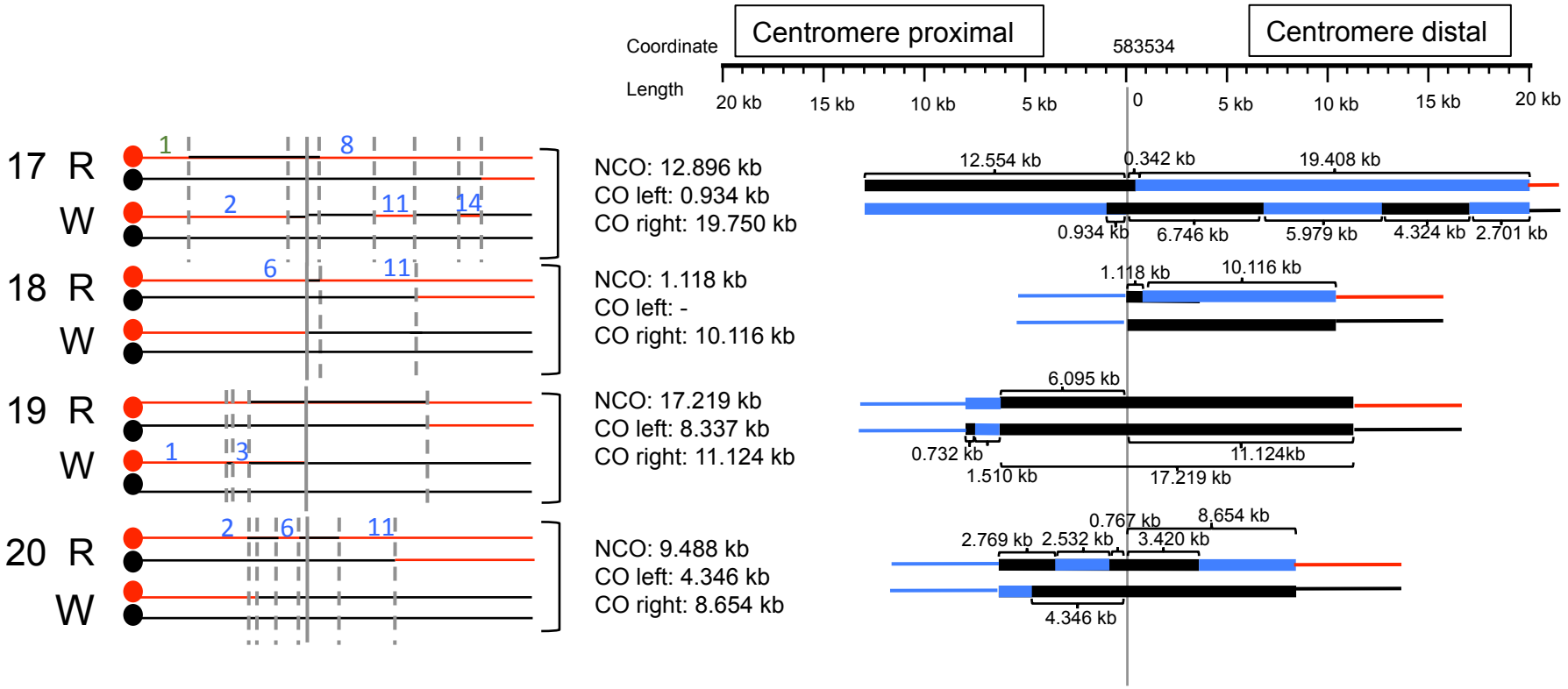


Figure S3. Chromosomes present in each sectored colony. The four chromosomes present within each sectored colony are diagrammed on the left. Red and black lines correspond to W303- and YJM-specific chromosomal segments, respectively, in CO and NCO products; red and black circles represent W303 and YJM centromeres, respectively. Solid gray vertical lines mark the position of the initiating DSB and dotted lines mark transitions between heterozygosity and homozygosity. Blue numbers correspond to primer pairs used to diagnose genetic linkages on the CO and NCO chromosomes within specific sectors (see Tables S2 and S3). On the right, the shorthand depiction is presented as in Figure 4, but 4:0 and 3:1 tract lengths are given and are presented to scale.

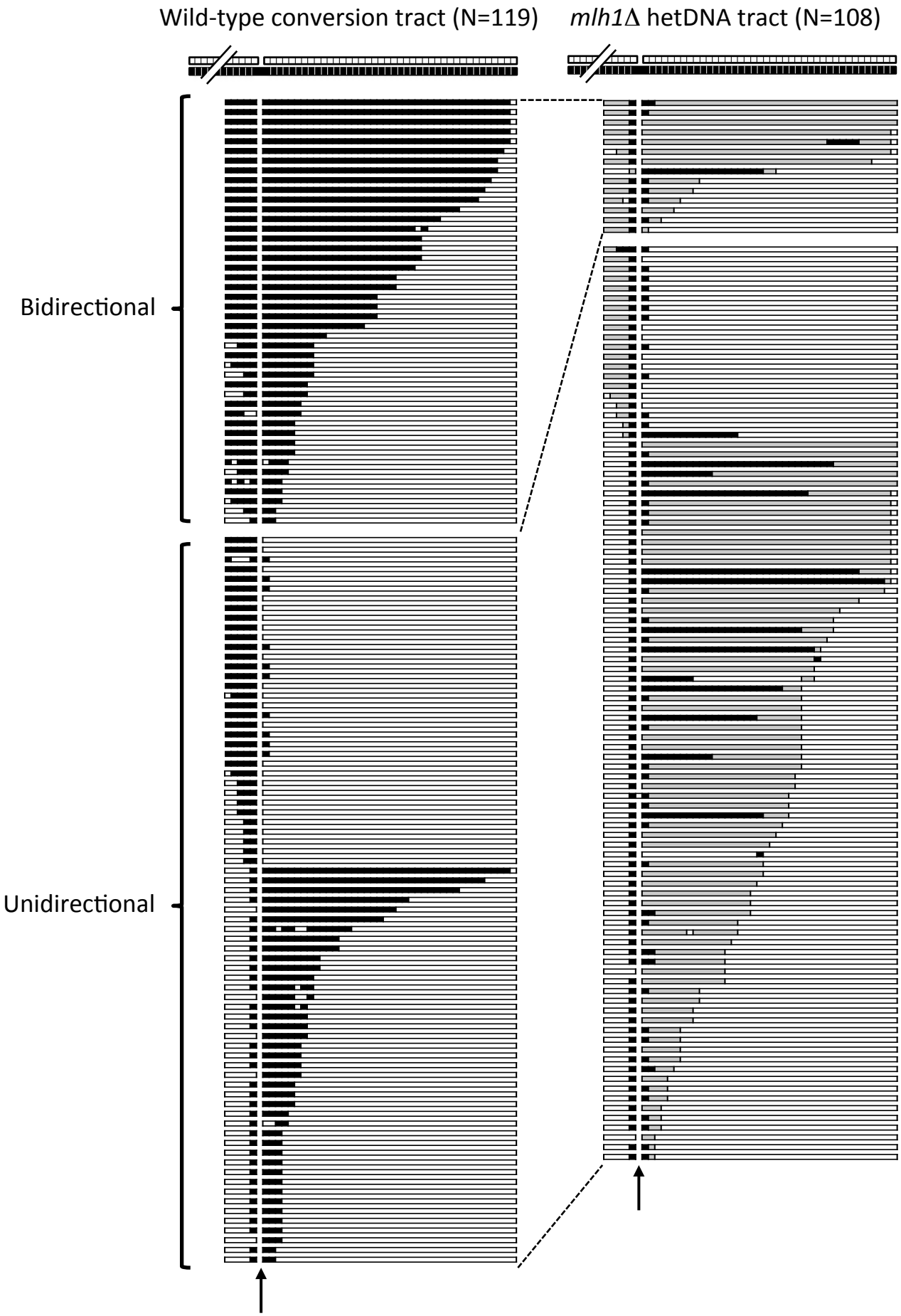

Wild-type conversion tract (N=119)
mlh1Δ hetDNA tract (N=108)
Bidirectional
Unidirectional

Figure S4. Contribution of MMR to NCO tract directionality in the ectopic system. The ectopic system shown in Figure 7A was used to map GC and hetDNA tracts in wild-type (adopted from Guo *et al*. 2017) and *mlh1Δ* strains, respectively. Each line represents an individual NCO event; white and black boxes represent recipient and donor SNPs, respectively, which were spaced at ~50 bp intervals. Grey boxes indicate hetDNA and vertical arrows mark the DSB position. Following the selection of Lys^+ recombinants, DNA was isolated from entire colonies without purification in order to preserve mismatches present in hetDNA intermediates. NCO tracts were truncated at the 5' end during PCR-amplification (only 5 upstream SNPs are represented) with barcoded primers. PCR fragments were pooled for SMRT sequencing and individual recombinants were then sorted by barcode. Conversion of first SNP adjacent to the DSB (8-nt from each of the I-*Sce*I generated 3' ends) has been shown to be due to proofreading activity of DNA polymerase δ (Guo *et al.* 2017) and was disregarded when determining tract directionality. For example, a tract with a long conversion tract (black box) upstream of DSB and a conversion tract covering only the first SNP downstream of DSB is considered a unidirectional tract.

Table S1: Yeast strains

Strain	Relevant genotype	Comments/reference
JSC12-1 (W3031A)	*MATa RAD5 ade2-1 leu2-3,112 his3-11,15 ura3-1 trp1-1 can1-100Δ::nat IV1510386::kanMX-can1-100*	Isogenic derivative of W303-1A
JSC21-1 (YJM789)	*MATα ade2-1 ura3 gal2 ho:hisG canΔ::NAT IV1510386::SUP4-o*	Isogenic derivative of YJM789
SJR4246 (W303)	*IV1510386::kanMX IV583526:CORE-UH*	CORE-UH cassette inserted between the *OCA6* and *DOS2* loci of JSC12-1
SJR4248 (YJM)	*IV1510386::SUP4-o IV583526:CORE-UH*	CORE-UH cassette inserted between the *OCA6* and *DOS2* loci of JSC21-1
SJR4250 (W303)	*IV1510386::kanMX IV583534:I-SceI*	CORE-UH of SJR4246 replaced with 18 bp I-*Sce*I cut site
SJR4252 (YJM)	*IV1510386::SUP4-o IV583534:I-SceInc*	CORE-UH of SJR4248 replaced with 22 bp mutant (non-cutting, nc) I-*Sce*I site
SJR4313 (W303/YJM)	*MATa/MATα IV1510386::kanMX/SUP4-o IV583534:I-SceI/I-SceInc*	SJR4250 x SJR 4252
SJR4315 (W303/YJM)	*MATa/matαΔ::URA3 IV1510386::kanMX/SUP4-o IV583534:I-SceI/I-SceInc*	*MATα* locus of SJR4313 replaced with *URA3* to prevent sporulation.
SJR4317 (W303/YJM)	*MATa/matαΔ::URA3 IV1510386::kanMX/SUP4-o IV583534:I-SceI/I-SceInc his3Δ::pGAL-I-SceI-hph/HIS3*	*his3-11,15* replaced with a *pGAL-I-SceI* fusion

Table S2. Primers and restriction site polymorphisms used to map GC tracts

Primer (Fig S1)		Primer name	Sequence (5' > 3')	Restriction Site	Restriction site presence (+) or absence (-)	
					W303	YJM
1	F	U567959F	CACCAACAGGTGTAGGCTCA	*NdeI*	-	+
	R	U568803R	TTTGCCATGGTTTATCAGGA			
2	F	U574454F	TCCAATCGTCATTGGTTTGA	*Hinf*I	-	+
	R	D574454R	ACTATCGGCGTCTTTGTCCA			
3	F	U574954F	CGGTATATTGTTTGAAGGCCC	*Aci*I	3 sites	2 sites
	R	D576563R	TCATACCTAAGAAGTCGTGAACAT			
4	F	U575934F	GCCCATTACTTAAGGCTCATATC	*Pst*I	-	+
	R	D576563R	TCATACCTAAGAAGTCGTGAACAT			
5	F	U579186F	TTGAAGAATTGAGGGAGCAA	*Bst*NI	-	+
	R	D579742R	GAACCAAGCTGGAGCATTTC			
6	F	U581508F	GGCCATTTGGGAGGAATATC	*Afl*II	-	+
	R	D582114R	TTTGCGAAAATGAGCAGACA			
7	F	OCA6F	CGCATCGTACTACAACATCGTATTC	*Bsr*G1	+	-
	R	DOS2R	CATCTTCGATACAAGCTACTTGTTC			
8	F	OCA6F	CGCATCGTACTACAACATCGTATTC	*Ssp*I	+	-
	R	DOS2+576R	TGTCGCCCTGTAAAATAGAACA			
9	F	U587100F	CATGATCATTGTCTGATTGCTG	*Bst*API	+	
	R	D587100R	GCAAAAATAAGGGTGCAATGA			
10	F	U588496F	GTCTTGGAGTGCGCAAAAA	*Bci*I	-	+
	R	D588694R	TGCTCACGAGCCATTGATAC			
11	F	D591116F	CAAACTTGCTATGAATGTCAGGTC	*Nar*I	-	+
	R	U592210R	TGTTGGCTTGAGAAACGTCA			
12	F	D593108F	TCGTCGCAGTAAGCATTGTC	*Aci*I	1 site	2 sites
	R	D594201R	CGTTCGGTTCTTGTTATTTCG			
13	F	D598460F	GGCAAGGTCGATCTACGAAA	*Xcm*I	-	+
	R	D599224R	AGTTACGATCGTCGCTGCTT			
14	F	U601761F	GGGCACTACCACCAAAGAAA	*Aci*I	2 sites	1 site
	R	D602336R	TATCGCACACTGGTTCTGCT			

Table S3. Linkage relationships on CO and NCO chromosomes in the same sector

Sector	Primer pairs	# P spores	# NP spores	Linkage
1R	6 and 11	2	49	NP
2R	6 and 11	2	40	NP
3R	2 and 8	0	53	NP
3W	6 and 8	22	0	P
4R	2 and 9	0	35	NP
6R	1 and 6	47	0	P
7R	1 and 9	24	2	P
8R[1]	2 and 9	49	3	P
	2 and 12	49	3	P
9R	3 and 8	32	0	P
10R[2]	1 and 4	0	33	NP
	1 and 6	33	0	P
	1 and 10	30	3	P
11R	2 and 9	23	4	P
12R	2 and 8	36	0	P
13R	2 and 5	16	0	P
	2 and 9	16	0	P
14R	6 and 9	45	1	P
14W	6 and 11	22	2	P
15R	2 and 6	24	0	P
	2 and 9	24	0	P
16R	2 and 10	1	23	NP
17R	1 and 8	28	0	P
17W	2 and 11	24	0	P
	2 and 14	24	0	P
18R	6 and 11	29	0	P
19W	1 and 3	46	0	P
20R	2 and 6	32	0	P
	2 and 11	32	0	P

Parental (P) refers to a W303-W303 or YJM-YJM relationship between the amplified fragments. In non-parental (NP) spores, there is a W303-YJM linkage between fragments. Primer pairs used to amplify fragments are given in Table S2 and their positions are shown in Figure S1.
[1]Fragment produced with primer pair 12 was sequenced to confirm transition point
[2]Single SNP at coordinate 575520 is homozygous