Supplementary Table 1. Oligonucleotides used in this study.

Name	Description	Size	Structure from 5'	Sequence
a	80STUECOV.12D	80	[D 34]-ins::D 12-[D 34]	5'-TGTTAGGTGCTGTGGGTGGTCCTAAATGGGGTAC-AGGCCTGATATC-CGGTAGTGTTAGACCTGAACAAGGTTTACTAAAA
b	80STUECOV.4X3	80	[D 34]-ins::D 4,R 4,D 4-[D 34]	5'- TGTTAGGTGCTGTGGGTGGTCCTAAATGGGGTAC-AGGC-CUGA-TATC-CGGTAGTGTTAGACCTGAACAAGGTTTACTAAAA
c	80STUECOV.6R6D	80	[D 34]-ins::R 6,D 6-[D 34]	5'- TGTTAGGTGCTGTGGGTGGTCCTAAATGGGGTAC-**AGGCCU**-GATATC-CGGTAGTGTTAGACCTGAACAAGGTTTACTAAAA
d	80STUECOV.12R	80	[D 34]-ins::R 12-[D 34]	5'- TGTTAGGTGCTGTGGGTGGTCCTAAATGGGGTAC-**AGGCCUGAUAUC**-CGGTAGTGTTAGACCTGAACAAGGTTTACTAAAA
e	74STU.6D	74	[D 34]-ins::D 6-[D 34]	5'-TGTTAGGTGCTGTGGGTGGTCCTAAATGGGGTAC-AGGCCT-CGGTAGTGTTAGACCTGAACAAGGTTTACTAAAA
f	74STU.6R	74	[D 34]-ins::R 6-[D 34]	5'- TGTTAGGTGCTGTGGGTGGTCCTAAATGGGGTAC-**AGGCCU**-CGGTAGTGTTAGACCTGAACAAGGTTTACTAAAA
g	LEU2.80e	80	[D 40]-[D 40]	5'-GCCGTTTTGTTAGGTGCTGTGGGTGGTCCTAAATGGGGTA-CCGGTAGTGTTAGACCTGAACAAGGTTTACTAAAAATCCG
h	RLEU2.80e	80	[R 40]-[R 40]	5'-**GCCGUUUUGUUAGGUGCUGUGGGUGGUCCUAAAUGGGGUA-CCGGUAGUGUUAGACCUGAACAAGGUUUACUAAAAAUCCG**
i	RLEU2.80f	80	[R 40]-[R 40]	5'-**CGGAUUUUUAGUAAACCUUGUUCAGGUCUAACACUACCGG-UACCCCAUUUAGGACCACCCACAGCACCUAACAAAACGGC**
j	LEU2B.80e	80	[D 38]-D 2-[D 40]	5'-GCCGTTTTGTTAGGTGCTGTGGGTGGTCCTAAATGGGG-AT-CCGGTAGTGTTAGACCTGAACAAGGTTTACTAAAAATCCG
k	RLEU2B.80e	80	[R 38]-R 2-[R 40]	5'-**GCCGUUUUGUUAGGUGCUGUGGGUGGUCCUAAAUGGGG-AU-CCGGUAGUGUUAGACCUGAACAAGGUUUACUAAAAAUCCG**
l	RLEU2B.80f	80	[R 40]-R 2-[R 38]	5'-**CGGAUUUUUAGUAAACCUUGUUCAGGUCUAACACUACCGG-AU-CCCCAUUUAGGACCACCCACAGCACCUAACAAAACGGC**
k.45	RLEU2B.e45	45	[R 22]-[R 23]	5'-**GUGGGUGGUCCUAAAUGGGG-AU-CCGGUAGUGUUAGACCUGAACAA**
l.45	RLEU2B.f45	45	[R 23]-[R 22]	5'-**UUGUUCAGGUCUAACACUACCGG-AU-CCCCAUUUAGGACCACCCAC**
m	5D3RLEU2B.80e	80	[D 38]-D 2-[D 5 R 35]	5'-GCCGTTTTGTTAGGTGCTGTGGGTGGTCCTAAATGGGG-AT-CCGGT**AGUGUUAGACCUGAACAAGGUUUACUAAAAAUCCG**
n	5R3DLEU2B.80e	80	[R 38]-R 2-[R 5 D 35]	5'-**GCCGUUUUGUUAGGUGCUGUGGGUGGUCCUAAAUGGGG-AU-CCGGU**AGTGTTAGACCTGAACAAGGTTTACTAAAAATCCG
o	5R3D20LEU2B.80e	80	[R 38]-R 2-[R 20 D 20]	5'-**GCCGUUUUGUUAGGUGCUGUGGGUGGUCCUAAAUGGGG-AU-CCGGUAGUGUUAGACCUGAA**CAAGGTTTACTAAAAATCCG
p	80STUECOV.12chiNR	80	[R 34]-ins::R 12-[D 34]	5'-**UGUUAGGUGCUGUGGGUGGUCCUAAAUGGGGUAC-AGGCCUGAUAUC**-CGGTAGTGTTAGACCTGAACAAGGTTTACTAAAA
q	DT_DLEU2B.60e	80	[D 28]-D 2-[D 30]-D 20 tail	5'-TAGGTGCTGTGGGTGGTCCTAAATGGGG-AT-CCGGTAGTGTTAGACCTGAACAAGGTTTAC-CCAGCGGAGTCCTTGGCCTA
r	DTRLEU2B.60e	80	[R 28]-R 2-[R 30]-D 20 tail	5'-**UAGGUGCUGUGGGUGGUCCUAAAUGGGG-AU-CCGGUAGUGUUAGACCUGAACAAGGUUUAC**-CCAGCGGAGTCCTTGGCCTA
r3	D3tRLEU2B.60e	63	[R 28]-R 2-[R 30]-D 3 tail	5'-**UAGGUGCUGUGGGUGGUCCUAAAUGGGG-AU-CCGGUAGUGUUAGACCUGAACAAGGUUUAC**-TAA
s	DTRLEU2B.60f	80	[R 30]-R 2-[R 28]-D 20 tail	5'- **GUAAACCUUGUUCAGGUCUAACACUACCGG-AU-CCCCAUUUAGGACCACCCACAGCACCUA**-CCAGCGGAGTCCTTGGCCTA
t	DT_DLEU2B.60f	80	[D 30]-D 2-[D 28]-D 20 tail	5'- GTAAACCTTGTTCAGGTCTAACACTACCGG-AT-CCCCATTTAGGACCACCCACAGCACCTA-CCAGCGGAGTCCTTGGCCTA
u	45DLEU2B.e	45	[D 38]-D 2-[D 5]	5'-GCCGTTTTGTTAGGTGCTGTGGGTGGTCCTAAATGGGG-AT-CCGGT
R.w	72TR6.e	72	[D 33 R 3]-R 1-[R 2 D 33]	5'-AAGAGAGTTGGAAAAGGGTTTTGATGAAGCTGT**CGC-G-GAT**CCCACATTCTGGGAAGACTTCAAATCCTTGTA
R.c	72TR6.f	72	[D 33 R 2]-R 1-[R 3 D 33]	5'-TACAAGGATTTGAAGTCTTCCCAGAATGTG**GGAUC-C-GCG**ACAGCTTCATCAAAACCCTTTTCCAACTCTCTT
D.w	72TD6.e	72	[D 36]-D 1-[D 35]	5'-AAGAGAGTTGGAAAAGGGTTTTGATGAAGCTGTCGC-G-GATCCCACATTCTGGGAAGACTTCAAATCCTTGTA
D.c	72TD6.f	72	[D 35]-D 1-[D 36]	5'-TACAAGGATTTGAAGTCTTCCCAGAATGTGGGATC-C-GCGACAGCTTCATCAAAACCCTTTTCCAACTCTCTT
T1	TRP5B.80f	80	[D 39]-D 1-[D 40]	5'-AGAATACAAGGATTTGAAGTCTTCCCAGAATGTGGGATC-C-GCGACAGCTTCATCAAAACCCTTTTCCAACTCTCTTAGAC
T2	ChiB.f1	80	[R 39]-R 1-[R 5 D 35]	5'-**AGAAUACAAGGAUUUGAAGUCUUCCCAGAAUGUGGGAUC-C-GCGAC**AGCTTCATCAAAACCCTTTTCCAACTCTCTTAGAC
T3	DT_DTRP5B.60f	80	[D 29]-D 1-[D 30]-D 20 tail	5'-GATTTGAAGTCTTCCCAGAATGTGGGATC-C-GCGACAGCTTCATCAAAACCCTTTTCCAAC-CCAGCGGAGTCCTTGGCCTA
T4	DT_RTRP5B.60f	80	[R 29]-R 1-[R 30]-D 20 tail	5'-**GAUUUGAAGUCUUCCCAGAAUGUGGGAUC-C-GCGACAGCUUCAUCAAAACCCUUUUCCAAC**-CCAGCGGAGTCCTTGGCCTA

Name	Description	Size	Structure from 5'	Sequence
P	Primer25	25	D 25	5'-ACCTTGTTCAGGTCTAACACTACCG
I	45.D	45	D 45	5'-TGGGGTACAGGCCTGATATCCGGTAGTGTTAGACCTGAACAAGGT
II	45.DR4D	45	D 12-R 4-D 29	5'-TGGGGTACAGGC-**CUGA**-TATCCGGTAGTGTTAGACCTGAACAAGGT
III	45.RD2	45	R 16-D 29	5'-**UGGGGUACAGGCCUGA**-TATCCGGTAGTGTTAGACCTGAACAAGGT
IV	45.RD1	45	R 20-D 25	5'-**UGGGGUACAGGCCUGAUAUC**-CGGTAGTGTTAGACCTGAACAAGGT
V	45.R	45	R 45	5'-**UGGGGUACAGGCCUGAUAUCCGGUAGUGUUAGACCUGAACAAGGU**
Dw	16_DOWN	16	D 16	5'-TCAGGCCTGTACCCCA

The structure of the oligonucleotides used in this work is schematized from the 5' end, where DNA sequences (D) are in blue and RNA (R) in red. Bases with homology to the chromosomal DSB ends are indicated in brackets and underlined. Insertions are indicated as "ins::". A dash separates the different parts of the oligonucleotides. The complete sequence of oligonucleotides is shown, where RNA sequences are in bold; when present, the restriction site/s that is introduced by the oligonucleotides is underlined (thin line for *Bam*HI, thick line for *Stu*I and double line for *Eco*RV). The DNA oligonucleotides were desalted (synthesized by Invitrogen, Carlsbad, CA); the RNA–containing oligonucleotides were desalted and deprotected (synthesized by Dharmacon, Lafayette, CO).

Oligonucleotides *P* to *Dw* were used for *in vitro* DNA synthesis experiments. The substrates for DNA synthesis were prepared by hybridizing a 25-mer oligonucleotide primer, ^{32}P labeled on the 5' end, to a 45-mer oligonucleotide template. To create the 4-nucleotide gap substrate a DNA 16-mer complementary to the 5' end of the RNA template was included in the hybridization reaction. All oligonucleotides used in DNA synthesis reactions were desalted, deprotected, if containing RNA, and PAGE-purified.

Supplementary Table 2. Transformation frequencies with DNA and RNA-containing oligonucleotides.

Name	Size	Structure from 5' end	Mutation*	nmoles	#†	Transformants per 10^7 cells‡	Verification¶
a. Repair of HO DSB in *leu2* (YPLac + GAL)							
		no oligo	NA	NA	35	<0.1 (0 – 0.2)	Seq. 16/16 ins or del
a	80	[D 34]-ins::D 12-[D 34]	ins::12 *Eco*RV+*Stu*I	1	10	220,000 (160,000 – 570,000)	Dig. *Stu*I 6/6; Dig. *Eco*RV 6/6; Seq 5/5 correct
b	80	[D 34]-ins::D 4,R 4,D 4-[D 34]	ins::12 *Eco*RV+*Stu*I	1	10	66,000 (60,000 – 130,000)	Dig. *Stu*I 6/6; Dig. *Eco*RV 6/6; Seq. 6/6 correct
c	80	[D 34]-ins::R 6,D 6-[D 34]	ins::12 *Eco*RV+*Stu*I	1	3	45,000 (31,000 – 52,000)	Dig. *Stu*I 5/6; Dig. *Eco*RV 6/6; Seq. 5/6 correct; 1/6 sub
d	80	[D 34]-ins::R 12-[D 34]	ins::12 *Eco*RV+*Stu*I	1	6	4,100 (620 – 8,000)	Dig. *Stu*I 7/8; Dig. *Eco*RV 8/8; Seq. 7/8 correct; 1/8 sub
e	74	[D 34]-ins::D 6-[D 34]	ins::6 *Stu*I	1	4	300,000 (220,000 – 350,000)	Dig. *Stu*I 5/6; Seq. 5/6 correct; 1/6 sub
f	74	[D 34]-ins::R 6-[D 34]	ins6 *Stu*I	1	7	19,000 (12,500 – 30,000)	Dig. *Stu*I 5/6; Seq. 5/6 correct; 1/6 del
g	80	[D 40]-[D 40]	/	1	8	182,000 (160,000 – 550,000)	
h	80	[R 40]-[R 40]	/	5	3	1.7 (0.9 – 2.6)	Seq. 3/4 correct; 1/4 sub
i	80	[R 40]-[R 40]	/	5	3	5.6 (5.2 – 6.1)	Seq. 12/12 correct
h + i		[R 40]-[R 40] + [R 40]-[R 40]	/	1	6	1 (0 – 8)	Seq. 5/5 correct
h + i		[R 40]-[R 40] + [R 40]-[R 40]	/	5	3	17.5 (6.1 – 23)	Seq. 6/6 correct

Name	Size	Structure from 5' end	Mutation*	nmoles	#†	Transformants per 10^7 cells‡	Verification¶
j	80	[D 38]-D 2-[D 40]	mmTA>AT *Bam*HI	1	3	230,000 (110,000 – 450,000)	
k	80	[R 38]-R 2-[R 40]	mmTA>AT *Bam*HI	1	3	0.5 (0 – 1)	
l	80	[R 40]-R 2-[R 38]	mmTA>AT *Bam*HI	1	6	0.6 (0 – 3.3)	Dig. *Bam*HI 2/2
k + l		[R 38]-R 2-[R 40] + [R 40]-R 2-[R 38]	mmTA>AT *Bam*HI	5	3	5.2 (2.6 – 13)	Seq. 16/18 correct; 2/18 sub
k.45 + l.45		[R 22]-[R 23] + [R 23]-[R 22]	mmTA>AT *Bam*HI	1	4	<0.1 (0 – 0)	
k.45 + l.45		[R 22]-[R 23] + [R 23]-[R 22]	mmTA>AT *Bam*HI	5	3	<0.1 (0 – 0)	
m	80	[D 38]-D 2-[D 5 R 35]	mmTA>AT *Bam*HI	1	3	160 (100 – 190)	Dig. *Bam*HI 8/8
n	80	[R 38]-R 2-[R 5 D 35]	mmTA>AT *Bam*HI	1	5	1,700 (1,300 – 2,100)	Dig. *Bam*HI 2/2
o	80	[R 38]-R 2-[R 20 D 20]	mmTA>AT *Bam*HI	1	3	46 (23 – 53)	
p	80	[R 34]-ins::R 12-[D 34]	ins::12 *Eco*RV+*Stu*I	1	3	76 (75 – 110)	Dig. *Stu*I 4/4 Dig. *Eco*RV 2/4; Seq. 2/4 correct; 2/4 sub
q	80	[D 28]-D 2-[D 30]-D 20 tail	mmTA>AT *Bam*HI	1	6	39,000 (22,000 – 60,000)	
q	80	[D 28]-D 2-[D 30]-D 20 tail	mmTA>AT *Bam*HI	5	3	40,000 (39,000 – 41,000)	
r	80	[R 28]-R 2-[R 30]-D 20 tail	mmTA>AT *Bam*HI	1	14	86.5 (60 – 200)	Seq. 16/16 correct
r	80	[R 28]-R 2-[R 30]-D 20 tail	mmTA>AT *Bam*HI	5	4	420 (250 – 620)	Dig. *Bam*HI 6/8

Name	Size	Structure from 5' end	Mutation*	nmoles	#†	Transformants per 10^7 cells‡	Verification¶
r3	63	[R 28]-R 2-[R 30]-D 3 tail	mmTA>AT *Bam*HI	5	3	0.9 (0 – 1.8)	
s	80	[R 30]-R 2-[R 28]-D 20 tail	mmTA>AT *Bam*HI	1	3	140 (120 – 155)	Dig. *Bam*HI 10/10
t	80	[D 30]-D 2-[D 28]-D 20 tail	mmTA>AT *Bam*HI	1	3	12,000 (10,000– 13,000)	
u	45	[D 38]-D 2-[D 5]	mmTA>AT *Bam*HI	1	3	78 (52 -94)	
b. Targeting to *leu2::HO* no DSB (YPLac w/o GAL)							
		no oligo	NA	NA	3	<0.1 (0 – 0)	
j	80	[D 38]-D 2-[D 40]	TA>AT *Bam*HI	1	3	100 (85 – 115)	
n	80	[R 38]-R 2-[R 5 D 35]	TA>AT *Bam*HI	1	3	13 (9 – 16)	
r	80	[R 28]-R 2-[R 30]-D 20tail	TA>AT *Bam*HI	1	3	<0.1 (0 – 0.3)	

Name	Size	Structure from 5' end	Mutation*	nmoles	#†	Transformants per 10^7 cells‡	Verification¶
c. Repair of I-*Sce*I DSB in *trp5* (YPLac + GAL)							
		no oligo	NA	NA	3	<0.1 (0 – 0)	
T1	80	[D 39]-D 1-[D 40]	mmG>C *Bam*HI	1	3	39,000 (34,000 – 51,000)	
T2	80	[R 39]-R 1-[R 5 D 35]	mmG>C *Bam*HI	1	9	28 (15 – 35)	Dig. *Bam*HI 13/14
T3	80	[D 29]-D 1-[D 30]-D 20 tail	mmG>C *Bam*HI	1	3	11,000 (10,000 – 16,000)	
T4	80	[R 29]-R 1-[R 30]-D 20 tail	mmG>C *Bam*HI	1	6	2.1 (1.5 – 4.7)	Dig. *Bam*HI 10/10

The structure of the oligonucleotides is shown from the 5' end, where DNA sequences (D) are in blue and RNA (R) in red. Bases with homology to the chromosomal DSB ends are indicated in brackets and underlined. Insertions are indicated as "ins::".

*Genomic change, relative to *LEU2* sequence, introduced by the oligonucleotides is indicated as "ins::" for insertion; 'mm' for mismatch. The numbers of base insertions, the kind of mismatch, as well as the new restriction sites that are generated are also indicated; "NA", not applicable; "/", no change.

†Number of repeats for each oligonucleotide transformation.

‡Median and confidence interval > 0.95, or alternatively the range (in parentheses) when number of repeated experiments was <6, of Trp^+ transformant colonies per 10^7 viable cells obtained by targeting **a,** oligonucleotides *a* to *u* to the HO DSB in *LEU2* after incubating cells in YPLac with 2% galactose; **b,** oligonucleotides *j*, *n* and *r* to the *leu2::HO* site without induction of the DSB after incubating cells in YPLac without galactose; and **c,** oligonucleotides *T1* to *T4* to the *I-Sce*I break associated with the CORE-I-*Sce*I cassette in *TRP5* after incubating cells in YPLac with 2% galactose. Survival was ~5% after transformation with or without oligonucleotides for wild type and for all mutant strains tested following 3 h of HO-DSB induction. Survival was ~50% following 4 h of I-*Sce*I-DSB induction and ~70% with no break induction.

¶The number of correctly modified transformant clones that were randomly chosen and verified by sequence analysis (Seq.) and/or by restriction digestion (Dig.) is indicated over the total number of clones tested; "ins or del" indicates base insertions or deletions, "sub" indicates a single-base substitution always confined within the region covered by the oligonucleotide sequence.

Supplementary Table 3. DSB-mediated stimulation of oligonucleotide targeting to the side of the break in a strand-dependent manner.

Position of DSB	Oligonucleotides	Trp^+ with no DSB	Trp^+ with DSB
10 kb upstream from *trp5::ins31*	***R.w***	0.8 ± 0.5	43 ± 9
	R.c	0.9 ± 0.3	440 ± 150
	D.w	28 ± 4	250 ± 110
	D.c	23 ± 2	1,200 ± 270
10 kb downstream from *trp5::ins31*	***R.w***	0.4 ± 0.1	51 ± 13
	R.c	0.2 ± 0.1	2.2 ± 1.3
	D.w	15 ± 6	150 ± 25
	D.c	7 ± 2	12 ± 6

Number of Trp^+ transformants per 10^7 viable cells resulting from targeting 1 nmole of oligonucleotides *R.w*, *R.c*, *D.w* or *D.c* following no induction or induction of a DSB (7 hours in galactose) that is either 10 kb upstream or 10 kb downstream from the *TRP5::ins31* locus. Presented are the mean and standard deviation for six experiments.

Supplementary Table 4. DSB repair with RNA-containing oligonucleotides in mutants of *RRP6* 3' exoribonuclease, RNase H and *RAD51*.

RNA-containing oligonucleotide		Mutant genotype	#	RNA transformation frequency in mutant*	RNA relative frequency†	DNA transformation frequency in mutant*	DNA Relative frequency†	DNA oligonucleotide
k	[R 38]-R 2-[R 40]	***rrp6***	4	0.2§ (0 – 1)	**0.2**	42,000 (37,000 – 56,000)	**0.2**	***j***
o	[R 38]-R 2-[R 20 D 20]	***rnh1***	4	75 (27 – 122)	**1.6**	250,000 (210,000 – 290,000)	**1.3**	***j***
r	[R 28]-R 2-[R 30]-D 20 tail	***rnh1***	7	90 (41 – 220)	**1.1**	36,800 (36,700 – 36,900)	**1**	***q***
r	[R 28]-R 2-[R 30]-D 20 tail	***rnh35***	3	36 (31– 58)	**0.4**	25,000 (24,000– 25,000)	**0.7**	***q***
r	[R 28]-R 2-[R 30]-D 20 tail	***rnh1 rnh35***	6	19 (12 – 20)	**0.2**	14,000 (13,000 – 15,000)	**0.4**	***q***
r	[R 28]-R 2-[R 30]-D 20 tail	***rad51***	4	160 (78– 250)	**1.8**	50,000 (22,000– 72,000)	**1.1**	***q***

"#" indicates the number of independent transformation experiments for each oligonucleotide in a given genotype.

*Median and range (in parentheses) of Leu^+ transformant colonies per 10^7 viable cells, resulting from targeting with 1 nmole of RNA-containing oligoncleotides or the corresponding DNA oligonucleotides to the HO-DSB in *LEU2*. Survival was ~5% after transformation with or without oligonucleotides for wild type and all mutant strains tested.

†Transformation frequency with RNA-containing or DNA oligonucleotides relative to the frequency in WT determined in the same series of experiments. All frequencies obtained for the RNA-containing oligonucleotides into the strains tested were either not statistically significantly different from WT (based on non-overlapping 95% confidence intervals) or resulted in a few-fold reduction or increase that paralleled the results with the corresponding DNA oligonucleotides in the same experiments. From each transformation experiment with RNA-containing oligonucleotides, six clones were subjected to B*am*HI digestion; in all cases the correct pattern was observed (not shown).

§Average.

Supplementary Table 5. DSB repair with RNA-containing oligonucleotides in mutants deleted for Ty regulatory genes or yeast non-essential DNA polymerase genes.

RNA-containing oligonucleotide		Mutant genotype	#	RNA transformation frequency in mutant*	RNA relative frequency†	DNA transformation frequency in mutant*	DNA Relative frequency†	DNA oligonucleotide
f	[D 34]-ins::R 6-[D 34]	*spt3*	4	6,850 (3,500 – 20,500)	**0.3**	53,000 (44,000 – 68,000)	**0.3**	*e*
d	[D 34]-ins::R 12-[D 34]	*spt3*	4	1,000 (930– 1,600)	**0.4**	47,000 (42,000– 53,000)	**0.3**	*a*
n	[R 38]-R 2-[R 5 D 35]	*spt3*	6	1,500 (1,400 – 1,650)	**0.5**	57,000 (33,000 – 60,000)	**0.4**	*j*
r	[R 28]-R 2-[R 30]-D 20 tail	*spt3*	9	45 (33 – 65)	**0.4**	120,000 (110,000 – 160,000)	**0.9**	*q*
f	[D 34]-ins::R 6-[D 34]	*est2*	3	36,000 (30,000 – 42,000)	**1.5**	190,000 (160,000 – 220,000)	**1.2**	*e*
n	[R 38]-R 2-[R 5 D 35]	*est2*	6	1,500 (730 – 2,200)	**0.5**	61,000 (29,000 – 110,000)	**0.4**	*j*
b	[D 34]-ins::D 4,R 4,D 4-[D 34]	*est1*	4	32,000 (16,000 – 43,000)	**0.5**	49,000 (30,000 – 110.000)	**0.3**	*a*
n	[R 38]-R 2-[R 5 D 35]	*est1*	4	130 (89 – 170)	**0.09**	150,000 (120,000 – 200,000)	**0.35**	*j*
b	[D 34]-ins::D 4,R 4,D 4-[D 34]	*pol4*	4	33,000 (29,000 – 37,000)	**0.6**	69,000 (59,000 – 76,000)	**0.4**	*a*
n	[R 38]-R 2-[R 5 D 35]	*pol4*	4	280 (260 – 330)	**0.2**	330,000 (230,000 – 76,000)	**0.7**	*j*
b	[D 34]-ins::D 4,R 4,D 4-[D 34]	*rev1*	4	25,000 (18,000 – 27,000)	**0.4**	38,000 (34,000 – 60,000)	**0.2**	*a*
n	[R 38]-R 2-[R 5 D 35]	*rev1*	4	560 (300 – 670)	**0.4**	360,000 (260,000 – 490,000)	**0.8**	*j*
b	[D 34]-ins::D 4,R 4,D 4-[D 34]	*rev3*	4	35,000 (32,000 – 37,000)	**0.6**	140,000 (74,000 – 150,000)	**0.8**	*a*
b	[D 34]-ins::D 4,R 4,D 4-[D 34]	*rad30*	4	67,000 (61,000 – 81,000)	**1.1**	140,000 (120,000 – 190,000)	**1.2**	*a*
d	[D 34]-ins::R 12-[D 34]	*rad30*	4	780 (680 – 880)	**1.0**	140,000 (120,000 – 190,000)	**1.2**	*a*
b	[D 34]-ins::D 4,R 4,D 4-[D 34]	*trf5*§	4	100,000 (92,000 – 150,000)	**1.7**	250,000 (210,000 – 360,000)	**2.2**	*a*
d	[D 34]-ins::R 12-[D 34]	*trf5*	4	1,800 (950 – 2,300)	**2.3**	250,000 (210,000 – 360,000)	**2.2**	*a*
n	[R 38]-R 2-[R 5 D 35]	*mip1*‡ (glu)	3	3.1 (1.2 – 3.7)	**10.3**	190 (175 – 210)	**1.4**	*j*

RNA-containing oligonucleotide		Mutant genotype	#	RNA transformation frequency in mutant*	RNA relative frequency†	DNA transformation frequency in mutant*	DNA Relative frequency†	DNA oligonucleotide
b	[D 34]-ins::D 4,R 4,D 4-[D 34]	***rev1 rev3***	6	64,000 (41,000 – 76,000)	**1.3**	132,000 (120,000 – 175,000)	**1.4**	***a***
b	[D 34]-ins::D 4,R 4,D 4-[D 34]	***rev1 rad30***	6	63,000 (45,000 – 79,000)	**1.2**	134,000 (100,000 – 150,000)	**1.4**	***a***
b	[D 34]-ins::D 4,R 4,D 4-[D 34]	***rev3 rad30***	6	55,000 (45,000 – 91,000)	**1.1**	120,000 (110,000 – 160,000)	**1.3**	***a***
b	[D 34]-ins::D 4,R 4,D 4-[D 34]	***rev1 rev3 rad30***	6	52,000 (44,000 – 57,000)	**1.0**	120,000 (86,000 – 180,000)	**1.3**	***a***

"#" indicates the number of independent transformation experiments for each oligonucleotide in a given genotype.

*Median and range (in parentheses) of Leu^+ transformant colonies per 10^7 viable cells, resulting from targeting with 1 nmole of RNA-containing oligoncleotides or the corresponding DNA oligonucleotides to the HO-DSB in *LEU2*. Survival was ~5% after transformation with or without oligonucleotides for wild type and all mutant strains tested.

†Transformation frequency with RNA-containing or DNA oligonucleotides relative to the frequency in WT determined in the same series of experiments. All frequencies obtained for the RNA-containing oligonucleotides into the strains tested were either not statistically significantly different from WT (based on non-overlapping 95% confidence intervals) or resulted in a few-fold reduction or increase that paralleled the results with the corresponding DNA oligonucleotides in the same experiments. 6/6 independent Leu^+ clones from each transformation with RNA-containing oligonucleotides in *spt3* and *est2* strains and 5/5 in the double and triple *rev1*, *rev3* and *rad30* mutant strains showed correct *Eco*RV or B*am*HI digestion (not shown).

§*TRF5* is poly (A) polymerase, with disputed role as a sigma DNA polymerase is homolog of *TRF4* (POLS) with potential DNA polymerase activity[30].

‡In the *mip1* mutant the DSB could not be induced in galactose, therefore, the experiment was done in glucose that is optimal condition for growth for this mutant. *Bam*HI site was present in 14/14 *mip1* Leu^+ transformants from the *n* oligonucleotide. Since recombination frequency with *n* did not show decrease relative to WT, the reason for the factor of 10 increase detected was not investigated further.

Supplementary Notes

Supplementary References

30. Wang, Z., *et al.* Structure/function analysis of the *Saccharomyces cerevisiae* Trf4/Pol sigma DNA polymerase. *Genetics* **160**, 381 (2002).

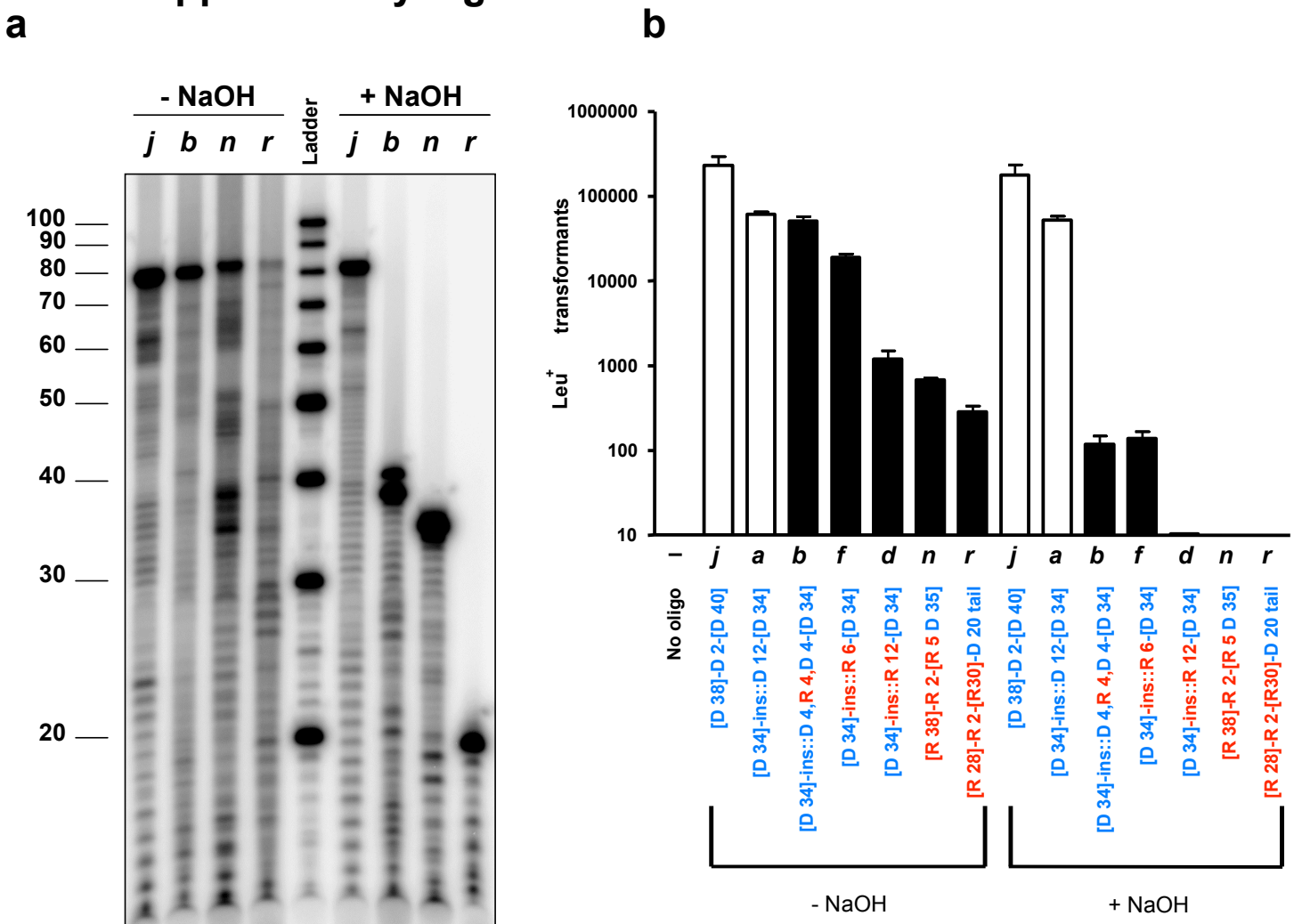
Supplementary Methods

Yeast strains. The yeast haploid strains used in this work are: YFP17[18] (*leu2*::*HOcs*, Δ*mat***a**::*hisG*, Δ*ho*, Δ*hml*::*ADE1*, Δ*hmr*::*ADE1*, *ade1*, *leu2-3*,*112*, *lys5*, *trp1*::*hisG*, *ura3-52*, *ade3*::*GAL*::*HO*) (FRO-767) with the HO cutting site in *LEU2* and FRO-1[17] (*MAT*α, *ade5-1*, *trp5*::I-*Sce*I-*CORE*, *his7-2*, *leu2-3*,*112*, *ura3-52*, *lys2-AluIR*), which contains the CORE-I-*Sce*I cassette (I-*Sce*I gene under *GAL1* promoter, the *hygMX4* hygromycin-resistance gene, the counterselectable *KlURA3*) and the I-*Sce*I cutting site in *TRP5*, as well as an *Alu* inverted repeat sequence in *LYS2*. Single deletion mutant strains contain the *kanMX4* module in place of the chosen gene, while double and triple deletion mutants contain the *kanMX4* and *hygMX4*, or *kanMX4*, *hygMX4* and *natMX4*[17] modules, respectively, in place of the chosen genes, and they are all derivatives of FRO-767. In the diploid strains FRO-888 and FRO-897[17] (*MAT***a**/*MAT*α, *his3*Δ*1*/*his3*Δ*1*, *leu2*Δ*0*/*leu2*Δ*0*, *met15*Δ*0*/*MET5*, *LYS2*/*lys2*Δ*0*, *ura3*Δ*0*/*ura3*Δ, *trp5*::*ins31*/*trp5*::*LEU2*) one copy of chromosome VII contains a *TRP5* locus inactivated by a 31-bp frameshift insertion, *trp5*::*ins31*, plus a CORE-I-*Sce*I cassette with the I-*Sce*I cutting site that can generate a DSB either 10 kb upstream or downstream of the *TRP5* mutant site. In the second copy of chromosome VII the *TRP5* gene has been replaced with the *LEU2* gene. The Trp^+ phenotype can be restored by the oligonucleotides, while the intact copy of chromosome VII can provide a template for repair of the DSB.

DSB induction. Briefly, cells with the HO inducible system derivative of FRO-767 were grown at 30˚ C overnight to saturation in 50 ml of rich medium containing 2% lactic acid (YPLac). After adding 5 ml of 20% galactose the culture was incubated with vigorous

shaking at 30˚ C for 3 h to express *GAL1*-HO and induce a DSB. Cells from FRO-1, FRO-888, and FRO-897 were grown at 30˚ C in 5 ml of rich YPDA medium overnight. Then 1.5 ml of the overnight culture were transferred into 50 ml of synthetic complete medium containing 2% galactose and incubated with vigorous shaking at 30˚ C for 4 h (7 hours for experiments involving oligonucleotide targeting to the side of a DSB, as described in Fig. 2) to express *GAL1*-I-*Sce*I and induce a DSB.

Supplementary Figure 1



Supplementary Figure 1. Effect of alkali treatment on oligonucleotide transformation. To establish the presence of ribonucleotides, using alkaline sensitivity, 4 nmoles of oligonucleotides *j*, *a*, *b*, *f*, *d*, *n* and *r* were treated with 1 M NaOH at 65° C for 1 h, and then neutralized with HCl 1.2 M and Tris-HCl 1 M pH 7.4. Alternatively, 4 nmoles of the same oligonucleotides were suspended in water and Tris-HCl 1 M pH 7.4. **a,** 3 pmoles of oligonucleotides *j*, *b*, *n* and *r* either treated or not treated with alkali, were 5'-labelled with [γ–^{32}P]ATP (Ready-To-Go T4 Polynucleotide Kinase, Amersham Biosciences) together with 0.1 μg of a 20/100 Oligo Length Standard (IDT) and run in a 12% polyacrilamide gel. The corresponding oligonucleotide bands were visualized using a Molecular Dynamics PhosphorImager. **b,** Number of Leu^{+} transformants per 10^{7} viable cells resulting from targeting 1 nmole of oligonucleotides *j*, *a*, (DNA oligonucleotides, white bars) *b*, *f*, *d*, *n* and *r* (RNA-containing oligonucleotides, black bars) treated or not treated with NaOH. Presented are the mean and standard deviation from three to six independent experiments. The ability of the RNA-containing oligonucleotides to transfer information to chromosomal DNA during DSB repair was clearly not due to contamination with DNA oligonucleotides, as shown by the dramatic reduction (more than two orders of magnitude) in transformation frequencies after alkali treatment of the oligonucleotides. Oligonucleotides *R.w*, *R.c*, *D.w* and *D.c* were also treated the same way with NaOH and used in transformation experiments as described in Fig. 2b. While transformation of DNA oligonucleotides *D.w* and *D.c* was unaffected by NaOH treatment, RNA molecules *R.w* and *R.c* showed more than 500-fold reduction in targeting when treated with NaOH compared to untreated molecules (data not shown).