Methods (detailed).

Purification of yeast chromosomal DNA.

Strain JZ105 (*mat1M Δmat2,3::LEU2, ade6-210, leu1-32, ura4-D18, his2*) was used for all the experiments. Cells were grown in 50 ml YEA. The cells were harvested and resuspended in 0.5 ml 1 M Sorbitol, 0.1 M EDTA (pH 7.5) and Zymolase (ICN Biomedicals). The suspension was incubated for 1 h at 37°C, centrifuged and resuspended in 0.5 ml DNAzol (Invitrogen). 0.5 ml 100% ethanol was added, the mixture was centrifuged. The precipitate was resuspended in 50 mM Tris-HCl, pH 7.5, 10 mM EDTA, containing 100 μg/ml RNase A (Qiagen). After incubation at 37°C for 30 min, the DNA was extracted with phenol/chloroform, precipitated with ethanol and resuspended in 100 μl TE (pH 8.0).

Hydrolysis and ligation of oligonucleotides.

For the oligo ligation experiments, end-labelled ART26 (TTTATTTATTTTC-AATAACrUGCAGTGTAATATAAAT) was hydrolyzed either in 500 mM NaOH for 2 h at room temperature followed by neutralization with equimolar HCl, or digested with 2-3 U of RNase A (Sigma) for 1 h at 37°C in 50 mM Tris, pH 8.0, 10 mM EDTA. The hydrolysis was subsequently analysed on 10% denaturing polyacrylamide gel. The salt or RNase A were removed from the samples using G-25 column (Amersham) or by phenol extraction, respectively. 10 pmol of hydrolyzed ART26 were mixed with 50 pmol of pre-annealed LM1/ART27 (LM1: Phosphate-TTTTCCATAACCGAAAGTAGTGACAAGTGTTGGCCATGGAACAG; ART27: CACTACTTTCGGTTATGGAAAAAGTTATTGAAAATAAATAAA) duplex. Ligation was performed at 16°C overnight, in 25 μl total volume, using either 400 U

of T4 DNA ligase or 10 U of *E. coli* DNA ligase. Ligation product was loaded on G-25 column to remove the buffer. The sample was split in three, two aliquots were treated with NaOH or RNase A (see above). For the experiment with dephosphorylated oligo, ART26 was digested with RNase A (see above) and phenol extracted. 200 pmol of the hydrolyzed ART26 were treated with Antarctic phosphatase (NEB) at 37°C overnight in 20 μl total volume. The phosphatase was inactivated by heating to 70°C for 5 min, and the buffer removed using G-25 column. The hydrolyzed, dephosphorylated oligo was end-labelled with [γ^{32}P] ATP, and the linker ligation performed (see above). The ligation products were analysed on 10% denaturing polyacrylamide gel.

LM-PCR.

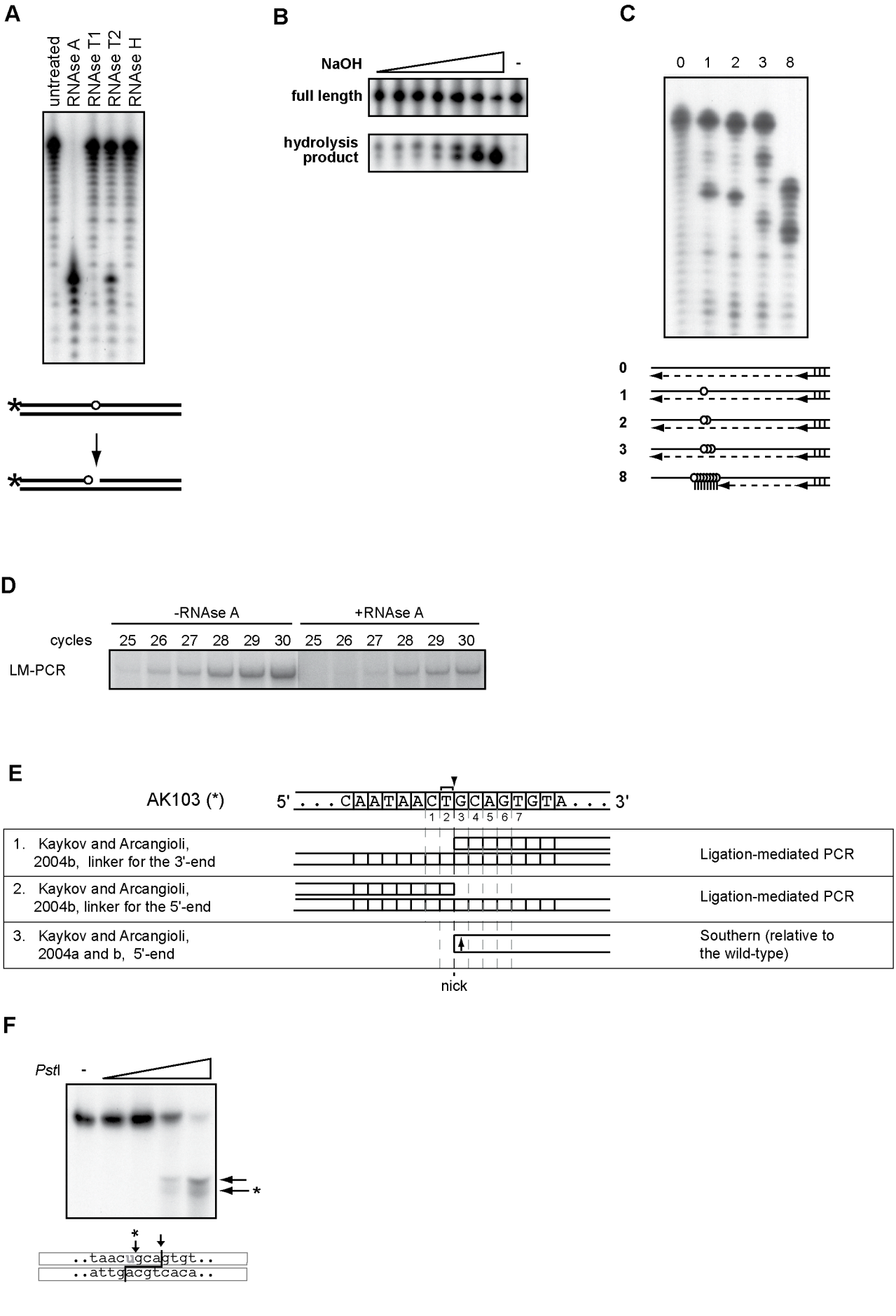
The DNA was denatured by incubation at 37°C for 20 min with 1/10 volume of 2 M NaOH, 2 mM EDTA, precipitated and re-dissolved in TE (pH 8.0). LM-PCR was done as described previously (Kaykov and Arcangioli, 2004) with the following modifications. The following oligos were used for linkers: LM1 (as above), LM1A (P-TTTTTCCATAACCGAAAGTAGTGACAAGTGTTGGCCATGGAACAG), LM2 (CACTACTTTCGGTTATGGAAAAAATTATTGAAAATAAATAAAAACG-AATATTC). Ligation was performed in 20 μl total volume at 16°C overnight, using 10 U of *E. coli* DNA ligase. The sample was split in two, and the volume was adjusted to 30 μl with TE (pH 8.0). 2-3 U of RNase A (Sigma) were added to one of the two fractions. After 5 hours of incubation at 37°C the volume of both treated and untreated samples was adjusted to 60 μl with TE (pH 8.0). 2 μl of the resulting solutions were used directly for LM-PCR. The PCR was performed using Taq DNA polymerase (NEB) and oligos LM3D (Kaykov and Arcangioli, 2004) and LM4

(CTGTTCCATGGCCAACACTTG) under the following conditions: 95°C (5 min); 15 to 34 cycles at 94°C (30 s)/65°C (30 s)/72°C (30 s). For the control PCR, oligos OK18 (Kaykov and Arcangioli, 2004) and mat1-NsiI-R (TTTGGGATGAGGTTTAATATATGTTTA) were used. PCR using these primers was performed as described above except primer annealing was done at 55°C.

Supplementary figure. A. The RNA bond at a single ribonucleotide in a DNA duplex is efficiently broken by RNase A. Oligo ART26 (TTTATTTATTTTCAATAACrUGCAGTGTAATATAAAT) was end-labelled and annealed to the oligo ART24 (ATTTATATTACACTGCAGTTATTGAAAATAA-TAAA). 4 pmol of the resulting duplex DNA was digested for 1 h at 37°C with RNase A (in 50 mM Tris, pH 8.0, 10 mM EDTA), or RNase T1 (in 50 mM Tris-HCl, pH 7.5, 2 mM EDTA), or RNase T2 (in 50 mM NaAc, pH 4.5, 2 mM EDTA), or RNase H (20 mM Tris-HCl, pH 7.5, 0.1 M KCl, 10 mM $MgCl_2$, 0.1 mM DTT, 5% Sucrose). RNase A was obtained from Sigma, RNases T1, T2 and H are from Invitrogen. The DNA was subsequently analysed on 10% denaturing polyacrylamide gel. The names of RNases used are given above the respective lanes. The line drawing below depicts the double-stranded nature and the hydrolysis of the substrate, used in the experiment. B. Hydrolysis of a single ribonucleotide bond in the DNA chain requires high concentrations of NaOH. 20 pmol of ART6 (TCAATAATrUTTTTTGTAATATAAATGTATAGTCTTTCTCCTTTGTTTTCTC TCGTTCGTTTCC) were treated for 2.5 h at 25°C with 20, 30, 40, 50, 100, 200 and 500 mM of NaOH. The reactions were neutralized with equimolar HCl, diluted and analyzed on a 10% denaturing polyacrylamide gel. C. Taq DNA polymerase can read

through ribonucleotides in template DNA. Oligos corresponding to the *mat1* sequence, containing none, 1, 2, 3 or 8 ribonucleotides (ART2: TCAATAATTTTTTTGTAATATAAATGTATAGTCTTTCTCCTTTGTTTTCTCT CGTTCGTTTCC, ART6 (see above), ART7: TCAATAATrUrUTTTTGTAATATA-AATGTATAGTCTTTCTCCTTTGTTTTCTCTCGTTCGTTTCC, ART4: TCAATA-ATTTrUrUrUTGTAATATAAATGTATAGTTTTCTCCTTTGTTTTCTCTCGTTCG TTTCC and ART5: TCAATArArUrUrUrUrUrUrUGTAATATAAATGTATAGTCT-TTCTCCTTTGTTTTCTCTCGTTCGTTTCC, respectively) were used as templates for primer extension. The number of ribonucleotides present in the templates is given above each lane. Only 8 ribonucleotides act as a complete barrier for the Taq polymerase. Annealing and primer extension were performed at 45°C for 45 seconds and 72°C for 30 seconds, respectively. 20 pmol of template oligo, 8 pmol of the end-labeled primer priSV2 (GGAAACGAACGAGAG) and 2 units Taq polymerase were used in the reaction. The extension products were analysed on a 6% denaturing polyacrylamide gel, followed by autoradiography. The schematic drawing below outlines the experiment. D. Linkers ligated to hydrolyzed imprinted DNA using T4 DNA ligase cannot be rehydrolyzed again using RNase A, as detected by PCR. Experiment was performed as described in the text, except the DNA was not pretreated with Antarctic phosphatase, and 400 U of T4 ligase was used in the linker ligation reaction. PCR amplification for untreated and RNase treated ligation products are shown. Number of cycles used in the PCR reactions is given. E. Ends at the imprint defined for the mutant strain AK103, where a *Pst*I restriction site is introduced at the site of the imprint by base substitution (Kaykov and Arcangioli, 2004; Kaykov et al., 2004). Description as for Fig. 1D. F. A. Kaykov and B. Arcangioli show that the nick, detected at the imprint of AK103 can be efficiently

ligated and subsequently cut by *Pst*I. We tested whether *Pst*I can cut a recognition site containing a single ribonucleotide at position 2 (see above). 10 pmol end-labelled oligo ART26 containing a single ribonucleotide, was annealed to the oligo ART24 of complementary sequence. The substrate was digested with 0.16, 0.8, 4 or 20 units of *Pst*I in 25 μl buffer for 2 hours at 37°C, and a fraction of each sample was analysed on a 20% native polyacrylamide gel. The molar concentration of sites in the restriction digest corresponds to digestion of 11.25 μg of λ DNA. The arrows to the right of the autoradiograph indicate restriction products. The arrow with an asterisk marks a product, where the overhang might have been shortened by RNA hydrolysis. The diagram below displays the sequence of the substrate, the position of the ribonucleotide and the sites of *Pst*I hydrolysis.


A
untreated
RNAse A
RNAse T1
RNAse T2
RNAse H
B
NaOH
-
full length
hydrolysis product
C
0 1 2 3 8
0
1
2
3
8
D
-RNAse A
+RNAse A
cycles
25 26 27 28 29 30 25 26 27 28 29 30
LM-PCR
E
AK103 (*)
5' . . . CAATAACTGCAGTGTA . . . 3'
1 2 3 4 5 6 7
1. Kaykov and Arcangioli, 2004b, linker for the 3'-end
Ligation-mediated PCR
2. Kaykov and Arcangioli, 2004b, linker for the 5'-end
Ligation-mediated PCR
3. Kaykov and Arcangioli, 2004a and b, 5'-end
Southern (relative to the wild-type)
nick
F
PstI
-
*
..taacugcagtgt..
..attgacgtcaca..