

SUPPLEMENTARY DATA

Table S1: List of all of the strains used in this study.

Figure S1: Pipeline of DRIPc-seq.

Figure S2: Metaplot analysis of RNase H-resistant DRIPc-seq signals in wild-type cells.

(a) Transcription units have been divided into promoter (TSS \pm 500 bp) or terminator (TTS \pm 500 bp) regions and the enrichment of RNase H-resistant DRIPc-seq signals in each region has been calculated by dividing the signal at each position by the mean signal found in the gene body (TSS to TTS). Genes have been clustered into 5 categories depending on their pattern of enrichment and the signals have been represented here as a heatmap. **(b)** Metaplot of the raw signals (left) or of the fold change (right) at promoter and terminator regions in the 5 clusters of genes.

Figure S3: RNase H-resistant DRIPc-seq signals are sensitive to the activity of the exosome.

(a) Principal Component Analysis (PCA) of the length of the DRIPc-seq peaks in the indicated genotypes. **(b)** Heatmap of the length of the DRIPc-seq signals relative to gene length. The colour key is indicated on the top left corner. A value of 0,6 indicates that 60% of the gene is covered by a DRIPc signal.

Figure S4: Class A R-loops are slightly longer than Class B R-loops.

Box-plot showing the distribution of sizes of R-loops in class A and class B R-loops (p-value obtained using the Wilcoxon Mann-Whitney test).

Figure S5 : R-loops associate with chromatin features characteristic of transcription elongation.

Barplot showing % intersect between Class A and Class B R-loops and the indicated chromatin factors. Colour indicates fold-enrichment for R-loop peaks vs. random expression-matched non-R-loop peaks (empirical p-value is shown).

Table S1: List of strains used in this study

Strain number	Mating type	genotype	provenance
LY112/LY113	h+/h-	leu1-32 ura4-D18 ade6-210	
LY2076/LY2077	h+/h-	leu1-32 ura4D? ade6+ ARS1::nmt1-RnhA::LEU2+	Legros et al., 2014
LY3360/LY3361	h+/h-	leu1-32 ura4D- ade6-210 rnh1Δ::hygR rnh201Δ::KanR	Legros et al., 2014
LY1052/LY1053	h-/h+	leu1-32 rrp6Δ::KanR	
LY117/LY118	h-/h+	leu1-32 ura4D? ade6-210 <i>dis3-54</i>	

Figure S1

DRIPc-seq protocol

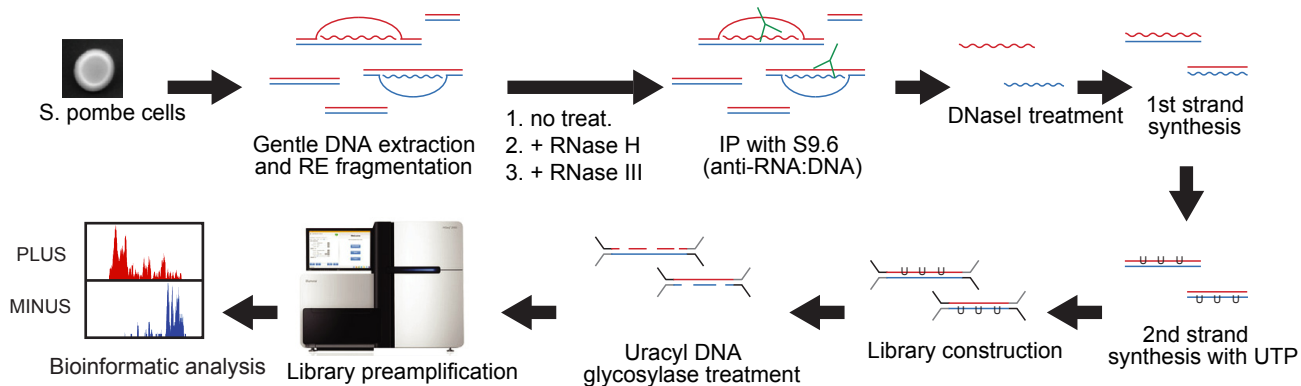
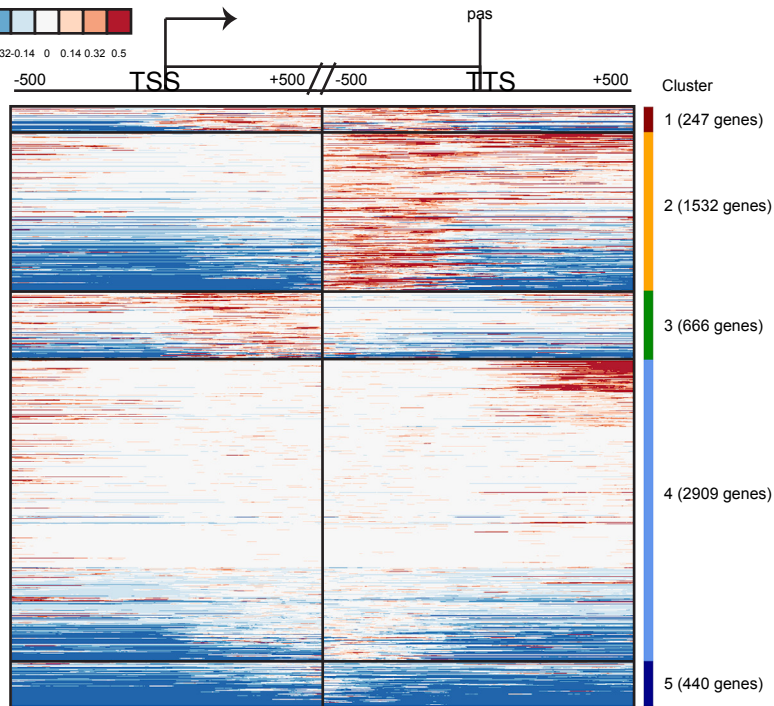


Figure S2

A

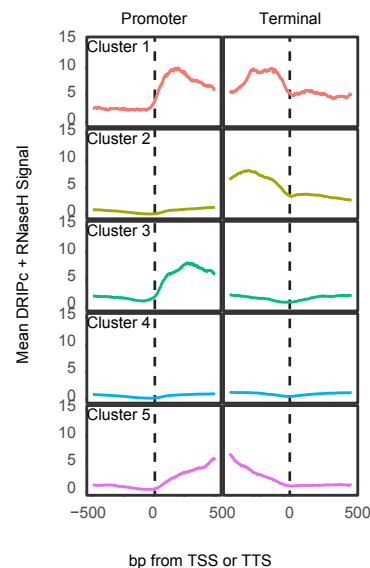
Heatmap of log₂ Fold Signal
(signal / mean genebody signal)

WT DRIPc + RNase H log₂ Fold
(signal / mean genebody signal)



B

Metaplot of
Raw DRIPc + RNase H Signal



Metaplot of log₂ Fold Signal
(signal / mean genebody signal)

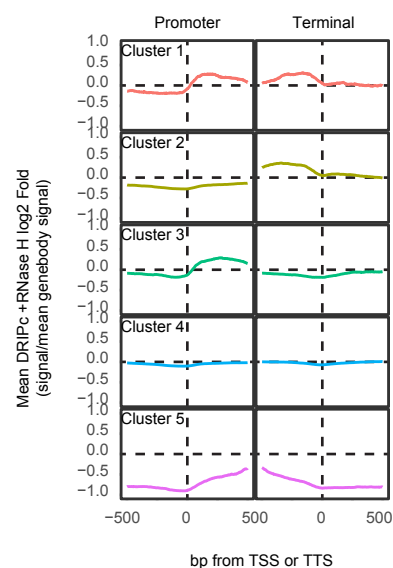
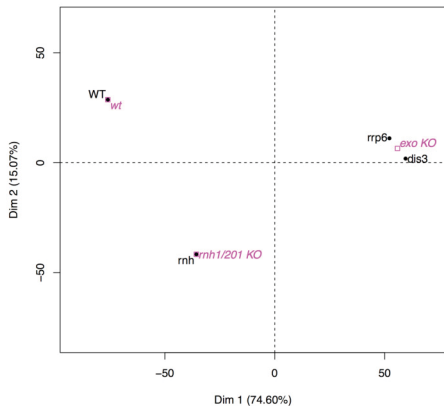


Figure S3

(a)



(b)

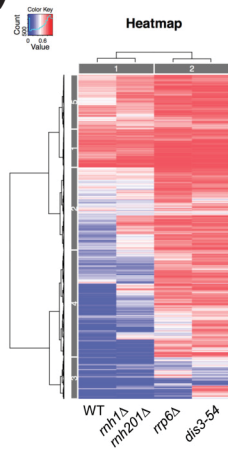


Figure S4

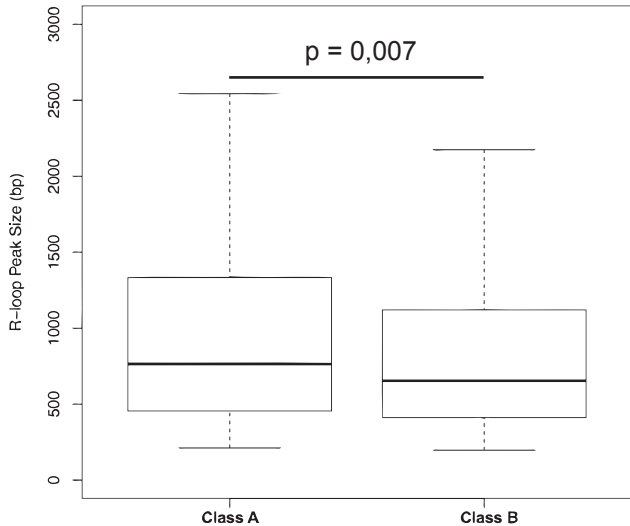


Figure S5

