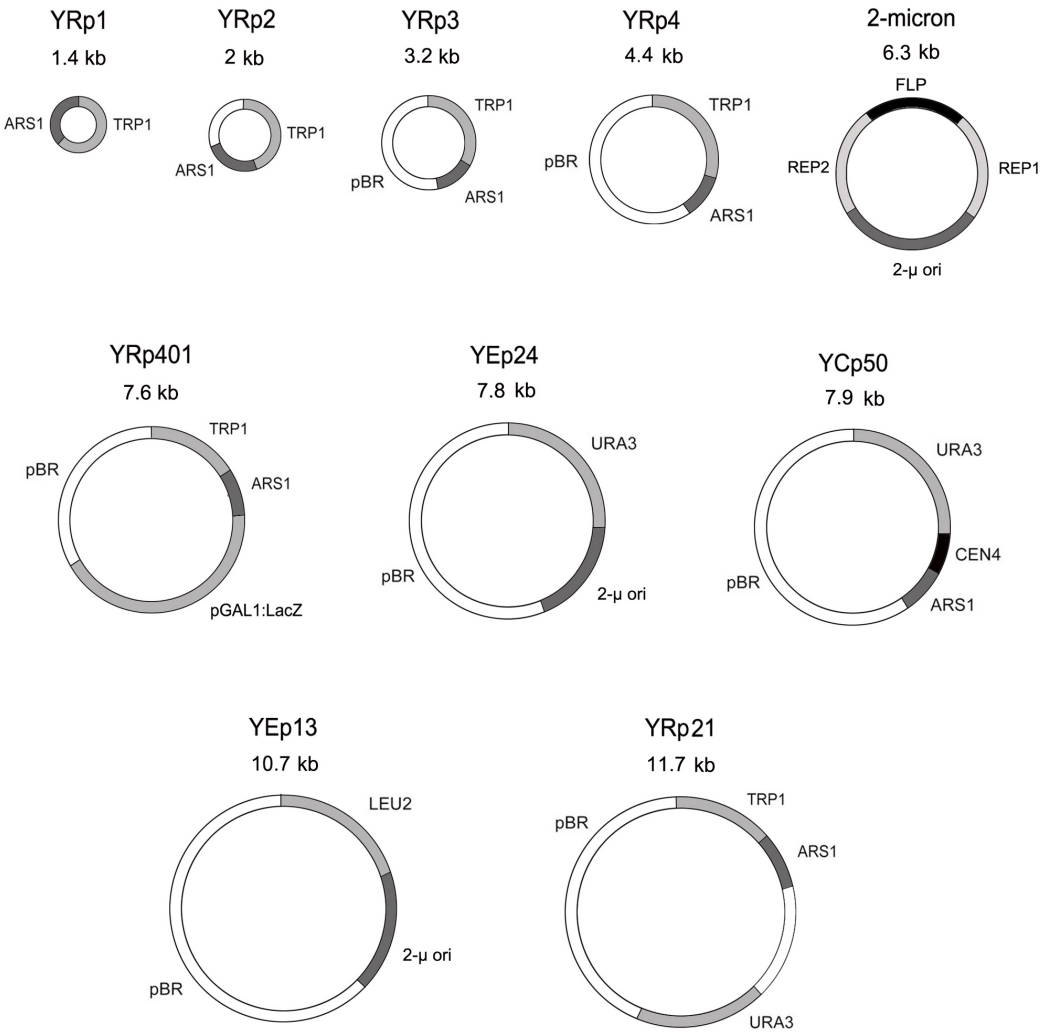
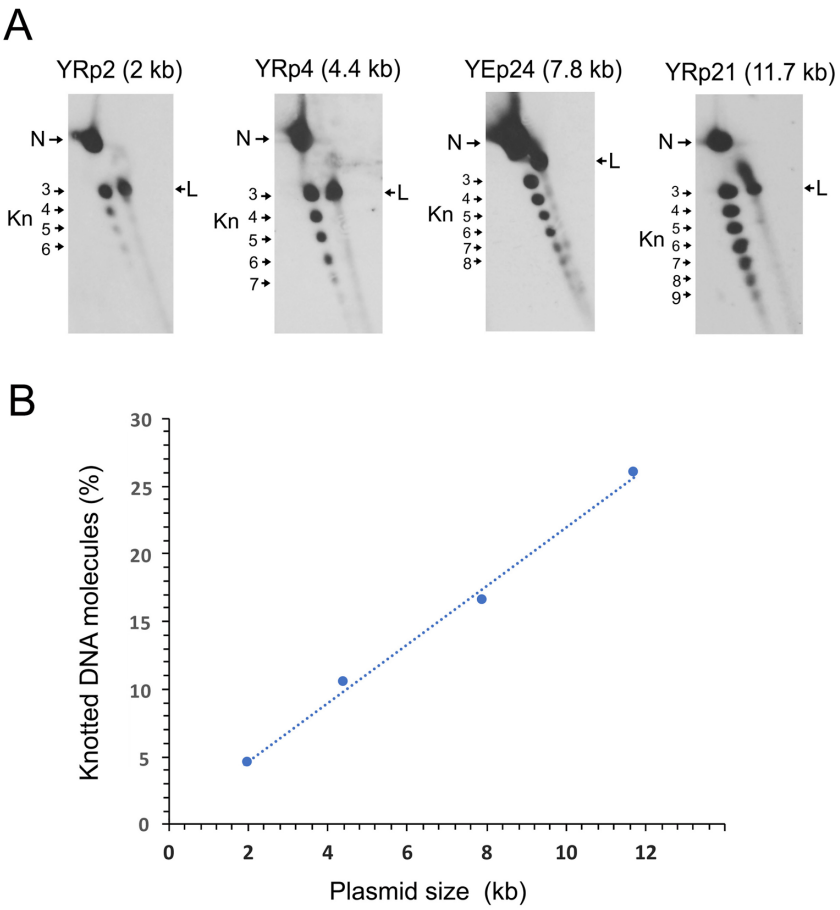
Supplementary Information



Supplementary Figure 1. Size and genetic configuration of yeast minichromosomes analyzed in the study. YRp3, YRp4, YRp401, YEp24, YCp50, YEp13 and YRp21 were amplified as bacterial plasmids in *Escherichia coli.* YRp1 and YRp2, which lack bacterial sequences, were constructed by circularization of linear DNA fragments generated via PCR amplification. Minichromosomes were transfected into *Saccharomyces cerevisiae* cells by electroporation. The 2-micron plasmid was endogenous in the yeast strains used.



Supplementary Figure 2. Dependence of topo II-mediated knotting of DNA on plasmid size. (A) DNA knots produced by topo II in bacterial plasmids YRp2 (2 kb), YRp4 (4.4 kb), YEp24 (7.8 kb), and YRp21 (11.7 kb). Reactions were done in 20 µl of 50 mM Tris-HCl pH 8, 1 mM EDTA, 50 mM NaCl, 10 mM $MgCl_2$, 7 mM 2-mercaptoethanol, 100 mg/ml of bovine serum albumin, and contained 10 ng of plasmid DNA and 10 ng of yeast topo II. Following incubation at 30ºC for 5 min, AMPPNP was added to 1 mM final concentration to effect DNA transport. Reactions were terminated after 10 min and each sample was phenol-extracted and ethanol precipitated. Recovered DNA samples were nicked with endonuclease BstNB1 (NEB) and examined in a two-dimensional agarose gel electrophoresis as described in Supplementary Table I. Gels were blotted and probed as described in the methods. Signals of nicked unknotted circles (N), linear DNA (L), and knots (Kn) with increasing number of crossings (3 to 9) are indicated. (B) The graph plots the fraction of knotted molecules (%) produced by plasmid size (kb).

DNA (Kb)	Electrophoresis of Lk distributions Agarose gel of 20 x 20 cm			Electrophoresis of DNA knots Agarose gel of 20 x 20 cm		
		Dimension I Chloroquine 0.6 μg/mL	Dimension II Chloroquine 3.0 μg/mL		Dimension I	Dimension II
	Agarose (w/v)	volts x time	volts x time	Agarose (w/v)	volts x time	volts x time
YRp1 (1.5 kb)	2 %	40V x 24h	100V x 4h	2 %	40V x 24h	200V x 3h
YRp2 (2 kb)	1.8 %	65V x 15h	100V x 4h	1.8 %	65V x 15h	165V x 3h
YRp3 (3.2kb)	1.2 %	65V x 15h	100V x 4h	1.2 %	65V x 15h	150V x 3h
YRp4 (4.4kb)	0.7 %	50V x 16h	70V x 4h	0.9 %	33V x 42h	150V x 3h
2-micron (6.3kb)	0.6 %	22V x 42h	90V x 4h	0.6 %	22V x 42h	125V x 3h
YEp24 (7.8 kb) YCp50 (7.9 kb)	0.6 %	22V x 42h	90V x 4h	0.45 %	22V x 42h	125V x 4h
YEp13 (10.7 kb) YRp21 (11.7kb)	0.4 %	20V x 42h	90V x 4h	0.4 %	33V x 42h	125V x 4h

Supplementary Table 1

Settings for 2D-gel electrophoresis

Chromosome	Length (Kb)	K^{P}_{CHR}	Kn 3	Kn 4	Kn 5	Kn 6	Kn 7	Kn 8
YRp1	**1,4**	**0,00**						
YRp2	**2,0**	**0,52**	0,52					
YRp3	**3,2**	**1,49**	1,20	0,21	0,06	0,02		
YRp4	**4,4**	**2,31**	1,72	0,39	0,12	0,05	0,02	0,01
2-micron	**6,3**	**2,62**	1,90	0,44	0,17	0,05	0,04	0,02
YEp24	**7,8**	**2,72**	1,95	0,46	0,18	0,08	0,04	0,01
YCp50	**7,9**	**2,79**	1,95	0,45	0,22	0,10	0,05	0,02
YEp13	**10,7**	**2,89**	2,10	0,40	0,23	0,09	0,05	0,02
YRp21	**11,7**	**2,93**	2,10	0,40	0,25	0,11	0,05	0,02

K^{P}_{CHR}: Total DNA knotting probability

Kn3 to **Kn8:** Probability (100 x) of DNA knot species of different number of crossings (3 to 8)

Supplementary Table 2

DNA knotting probability of yeast minichromosomes