TABLE 1. *Requirements for transformation by R-factor DNA*

DNA species	Transformants/μgDNA
R6-5 (F-like)	
Closed circular	9.2×10^4
+DNase (before)	<0.3
+DNase (after)	7.7×10^4
+RNase (before)	9.6×10^4
+Pronase (before)	8.1×10^4
Phenol extraction	9.9×10^4
Isolated from transformed bacteria	7.0×10^4
Catenated	3.2×10^4
Open-circular	5.6×10^4
Denatured	<0.3
Sonicated	<0.3
No DNA	*
No bacteria	<0.3
R64-11	
Closed circular	9.4×10^3
No DNA	*
No bacteria	<0.3

Transforming ability of different molecular forms of R-factor DNA was assayed (see *Methods* and Fig. 1). All R6-5 DNA species used in this experiment were isolated from *E. coli* minicells, and DNA concentration ranged from 0.6–5 μg/ml. The sample labeled *open-circular DNA* was obtained from peak 3 of a cesium chloride–ethidium bromide gradient containing R-factor DNA isolated from minicells and was free from significant contamination by (noncircular) chromosomal DNA (22). This fraction was composed of about 85–90% nicked (open) circular R-factor DNA, and 10–15% noncircular R-factor DNA molecules (23). Transformation efficiency was determined after a 120-min incubation in antibiotic-free medium. Where indicated, R-factor DNA preparations were incubated with pancreatic DNase (10 μg/ml), pancreatic RNase (20 μg/ml, Worthington), or Pronase (100 μg/ml, Calbiochem) at 37° for 5 min before use in the transformation assay. DNase treatment was done in the presence of 10 mM $MgCl_2$; the RNase preparations were previously heated to destroy DNase activity (6). Pronase was self-digested for 37° for 1 hr and at 80° for 2 min, and then chilled rapidly before use. Phenol extraction of DNA was done as described (7). The terms *before* and *after* refer to the period of incubation of R-factor DNA with $CaCl_2$-treated cells at 42°. Since the competence of bacteria varied somewhat in different experiments, the effects of R-factor DNA structure on transformation were determined with a single batch of $CaCl_2$-treated cells.

* No colonies were observed when 10^9 bacteria were assayed in the absence of DNA.