DNASEI PROTECTION

References:

Purpose and Comments: You will have first to determine the concentration of DNaseI that cleaves 30-50% of your input DNA. A rule of thumb is to multiply this amount by 10 to get the adequate concentration of enzyme required to digest the DNA in presence of nuclear extract. See also the alternate protocol

Material and solutions:
End-labelled DNA (labeled on ONE strand only!!)
DNaseI

Protocol:
1. End-label the probe and gel-purify it (this is the best solution to get rid of all free nucleotides).
2. Determine the concentration of DNaseI that yield 50% cleavage (try to cut 50-100 fmol, ca. 100000 cpm). Usually, one uses from 0.01 to 10 units DNaseI for 1'30'' at RT.
3. Proceed to DNaseI digestion in the GRA binding mixture:
   a. Perform your binding reaction as usually.
   b.If concentrations in $MgCl_2$ and $CaCl_2$ are below 5mM, bring the mixture to this concentration.
   c. Add DNaseI every 15 sec to each sample.
   d.Stop the reaction by adding an EDTA/EGTA mix that will yield a 10mM final concentration.
   e. Load the sample on a non denaturing acrylamide gel and run it for 2 to 3 hours at 4°C.
   f. Autoradiograph the gels and elute free and bound DNA (crush and soak method).
   g. Filter the polyacrylamide/buffer mix through a Millipore 0.45µm filter.
   h. Ethanol precipitate DNAs in presence of 10µg t-RNA and resolve on a sequencing gel (5 to 8%)

Alternate Protocol: If you can achieve conditions were at 60 to 75% of the input DNA is bound to the protein, it is not necessary to go through the GRA procedure. Just perform your binding reaction, treat with the appropriate DNaseI concentration and stop your reaction by adding 2v PCI, reextract once with CI and go through step h-.