DnaseI Footprinting

(Based upon the protocol from the Promega Core Footprinting Kit)

5x Binding Buffer (works for EBNA-1)
100mM HEPES
200mM KCl
5mM $MgCl_2$
5mM DTT
5mM EDTA
50% glycerol

Ca^{+2}/Mg^{+2} Solution
5mM $CaCl_2$
10mM $MgCl_2$

DnaseI Stop Solution
4ul 5M NaCl
6ul 0.5M EDTA pH 8.0
5ul 20% SDS
85ul dH_2O

Loading Solution
1:2 (v/v) 0.1M NaOH:formamide
0.1% xylene cyanol (ΞΨ)
0.1% bromophenol blue (BΦB)

Protocol:

1. Combine an appropriate concentration of probe that has been labeled on one strand (100-150 fmol probe has worked so far) with the appropriate concentration of protein in a total volume of 50ul at 1x Binding Buffer conditions.
   (e.g. order of addition: buffer, dH_2O, probe, protein).
   - [Protein]: have a negative control (no protein) for each category of digestion that you do (i.e. different DnaseI concentrations, digestion times)
   - A protein titration can be great to determine the right range of protein to work with and to possibly determine if there is a binding site of higher affinity than others.
2. Incubate reaction for at least 10min at 4°C-37°C depending on your situation (e.g. EBNA-1 can bind effectively to DNA within this range, I do the incubation on ice personally)
3. Dilute the DnaseI in ice-cold 10mM Tris-HCl (pH 8.0). A good working range is a dilution between 1:10 and 1:1000. (1:20 worked well for me)

NOTE: at this point, deal with two reactions at a time to avoid insanity and be accurate in your addition of the necessary solutions.

4. Add 50ul of $Ca^{+2}/MgCl^{+2}$ solution @ RT, incubate reactions at RT > 1min
5. Synchronize your timers! When working with two reactions at once, plan the digestions so that you can feasibly have 20-30 seconds per solution that you need to add between other additions. (e.g. If you are doing 2 digestions both for 60 seconds, start one reaction 30 seconds before the second to allow enough time for DnaseI and Stop Solution addition)
6. Add 3ul of diluted DnaseI solution, mix by pipetting up-and-down several times. Allow the reaction to continue for the pre-determined amount of time.
7. Add 90ul of the Stop Solution at RT-37°C and mix by pipetting up-and-down several times.
8. Repeat steps 4-7 until all reactions are digested and stopped.

9. Add 1/10 volume 3M NaOAc, mix by pipetting.
10. Add an equal volume of saturated phenol, vortex 20-45 sec.
11. Centrifuge @ 2000rpm, 2min to separate aqueous from organic.
12. Remove aqueous phase (top) to a fresh microfuge tube.
13. Purify probe, method will depend upon the size of the probe.
    - 25-125bp: Spin columns (e.g. Qiaquick Nucleotide Removal Kit) work well. Elute twice with 50ul warm dH_2O.
    - >125bp: Ethanol precipitation works well. Do not resuspend pellet after precipitation.
14. Lyophilize DNA by SpeedVac (centrifugation, vacuum, med/high heat)
15. Add 4-10ul Loading Solution to the elution/pellet. Mix by pipetting and store @ -20C until ready to run the gel.
16. When the urea-denaturing gel is poured and is being pre-run, heat the sample @ 80C to evaporate the aqueous content of the sample (~15-30min), or heat the sample @ 80C to heat denature the DNA of the already 10ul sample.
17. Load an appropriate amount of the 10ul to be able to visualize the signals by autoradiography (e.g. $1\text{-}1.5x10^6$ has worked well for me).