Electrophoretic Mobility Shift Assay (EMSA)
DTV version

Day 1: Labeling the probe

(typically label 0.5-1pmols and use 10fmol per sample)

Standard Reaction

0.5 pmol DNA (measured in ngs, for ~600bp fragment this is ~200ng)
4uL 10X T4 polynucleotide kinase buffer
1-2ul P32 ATP (enough to get at least 2 fold molar excess to DNA, isotope comes as 2 pmol/uL)
1uL T4 polynucleotide kinase
H20 to total 39uL

Mix all "cold" reagents together, add "hot" ATP last

37C for 30 mins, add another 1uL T4 PNK, 37C for 30 mins

Heat inactivate at 65C for 30 mins

Clean up the probe using Quiagen PCR purification kit
[be sure to aliquot out reagents from kit to use in the rad area…DON'T use the stocks directly]

Elute from probe from column 2X with 10uL dH20 each time

Day 1: Casting the gel

Wash both panes of glass with windex and 70% EtOH
Dry with KimWipe
Assemble with toothed spacers on sides, winged spacer on bottom
Clamp corners, bottom and side with binder clips
Lie on a slight angle (can use microfuge tube rack)

Native PAGE Preparation (4%)
Add in this order
63.25ml dH_2O
3.75ml 10x TBE
7.5ml 40% 37:5:1 acrylamide
0.5ml 10% APS
50ul TEMED

Pour gel using 60mL syringe (panes at a slight angle)

Lie panes flat after pouring

Place comb between the glass panes, do not insert completely to facilitate comb removal.

Add more gel solution every 5min as needed to keep gel volume up to the top of the comb until it solidifies.

Store the gel in saran wrap at 4C overnight

Day 2: Preparing the apparatus

Ensure you have enough 0.5x TBE

0.5x TBE:
50ml 10x TBE
950ml dH_2O

Remove winged spacer from bottom of gel and gel comb upon solidification and attach to the vertical gel apparatus, place sponges between the gaskets of apparatus and the gel plate. Push down on the sponges at the gasket junction with a 1000ul pipette tip to ensure a tight seal.

Bring gel apparatus to 7_{th} floor cold room and fill lower buffer well on an angle to prevent formation of an air bubble between the glass plates. Fill the upper buffer well above the top of the back plate. Use a 3ml syringe and small bore needle to wash each well with buffer to remove unpolymerized (liquid) acrylamide.
Run gel at 10-15 V/cm (200-300V for 20cm gel) for at least 45mins at 4C. This ensures that the entire gel is at the same temperature and that free radials formed during the polymerization of the gel are removed before sample separation. Check after 30 minutes of running for buffer leaks. Meanwhile:

Day 2: Protein/Probe incubation

4uL 5X EMSA buffer
1-2uL DNA probe (want ~10fmol)
1-5ul protein, diluted in **1x EMSA buffer** (typical ranges from 20-400ng, be sure to include a 0ng control)
2uL 10X polydIdC
H20 to total 20ul

Incubate protein with DNA for at least 5 mins at room temp before running gel [it's a good idea to add the protein last after all other reagents are added. Also, keep protein **on ice** until ready to use. For the 0ng protein controls, add 2-3ul Blue Juice as a loading visual aid]

Day 2: Sample Loading and Separation:

Turn off power supply.
Load each lane with gel loading tips or P10 tips to ensure all of the sample enters the well.

Run gel at 10-15 V/cm (200-300 V for 20 cm gel) until bromophenol blue marker contained in the free probe lane has migrated at least halfway through the gel (2-4hrs).

Just as the gel is about done running, erase the PhosphorImager Storage Screen by exposure to white light (use extended erase with the Screen Eraser in the gel camera room). Additionally, go get about 1.0-1.2lbs of dry ice and deposit it into the trap of the gel dryer and start pre-heating the gel dryer to 80C for at least 10 min.

Remove gel from apparatus and remove the smaller, back pane of glass. Be careful not to stretch the gel too badly as you do this. Cover the gel with a piece of Whatman paper and remove the gel from the second pane of glass. Cover the gel with a piece of Saran Wrap and place in the gel dryer underneath the plastic sheet of the gel dryer. Closer gel dryer cover and dry at 80C for about 1 hr.

Remove Whatman/Gel/SaranWrap from the gel dryer. Be careful not to pull off the Saran Wrap from the gel when lifting the plastic sheet of the gel dryer off.

Trim the Whatman/Gel/SaranWrap to the size of the gel.
Tape the Whatman/Gel/SaranWrap to a PhosphorImager Exposure Cassette, place the PhosphorImager Storage Screen on top and lock into place. Expose the screen o/n at RT (can minimally expose the screen for ~4 hrs to detect a signal if the radioisotope is fresh enough). Visualize the screen by PhosphorImager (STORM).