Cell Gel

Gardella, T., P. Medveczky, T. Sairenji, and C. Mulder. 1984. Detection of circular and linear herpesvirus DNA molecules in mammalian cells by gel electrophoresis. J. Virol. 50:248-254.

Prepare in advance:

1) 2200 mls 1X TBE at 4°C.
2) 400 mls 0.75% agarose in 1X TBE (no ethidium) at 55°C.
3) 50 mls 0.8% agarose/2% SDS in 1 x TBE at 55°C.
   - 44.5 ml 1X TBE
   - 5 ml 20% SDS
   - 0.5 ml 10X TBE
   - 1 mg/ml ~~pronase~~ added just before use

(Protease; Sigma P5147) XIV 19 => $30.00 Streptomyces griseus.

4) 10 mM phosphate buffer pH7.0
   - to make 1M phosphate buffer (100X)
     - Make 500 cc each of
     - Na_2HPO_4 dibasic MW 142.0 71 grams per 500 cc
     - NaH_2PO_4*H_2O monobasic MW 137.99 68.5 grams per 500 cc
   - Start with 200 cc of Na_2HPO_4 and pH with NaH_2PO_4 * H_2O to 7.0. Make 500 cc by adding both buffers to keep pH at 7.0.
5) TBE/Ficoll loading buffer
   - 15% Ficoll
   - 0.01% bromophenol blue
   - 1X TBE
   - 5 ul of RNAse added per ml just prior to use

Add 20ul RNaseA

6) lambda markers and marker for the probe (e.g. linearized DNA diluted in 2.5 ug/ml SS DNA)

For second day:

1) 500 cc of 0.2M HCl
   - 10 cc of concentrated HCl added to 490 cc of water
2) 500 cc of 1.5M NaCl and 0.5M NaOH
   - 44 grams NaCl
   - 10 grams NaOH
   - 500 cc water
3) 1 liter of 1M Tris pH 8.0 and 1.5M NaCl
4) 10X SSC
5) 100 cc of 0.4M NaOH
   - 4 ml of 10N NaOH to 100 cc of water
6) 200 mls of 2X SSC/0.2M Tris pH 8.0
   - 20 cc 20X SSC
   - 40 mls 1M Tris
7) Prehyb buffer
8) Probe

1. Assemble large gel rig with plug placed about an inch in from the top. Use tape around

plug to help get it out.
2. Pour 0.8% agarose into gel rig and allow to solidify. Place whole gel rig in cold.
3. Count cells. If viability <80% consider ficoll cells to reduce background. Aliquot 2-2.5 x 10^6 cells per lane.
4. Pellet cells with optional PBS wash. Keep pellets on ice and pour wells as cells are spinning. Lysing gel should be cold and used as quickly as possible to reduce diffusion of SDS.
5. Weigh out ~~pronase~~ Protease at 1 mg/ml and add to cooled lysing agarose.
6. Remove the plastic plug from the gel. Place medium width 15 well comb about 1/8" from bottom of gel and flush against front wall of the regular gel so that the majority of the SDS will run through the wells.
7. Pour lysing gel and place in cold room.
8. Wash out wells with TBE and load as soon as possible.
   Make sure to add lambda markers and a marker for the probe (100-500 pg)
9. Take 1 cc of TBE/Ficoll and add 5ul of 10 mg/ml RNAase A.
10. Resuspend pellets in 50-75 ul of TBE/Ficoll/RNAaseA and load.
11. Run at 15 volts in the cold for a minimum of 3-4 hours and then increase to 100 volts for at least 18 hours.

12. Transfer the whole gel (with lanes) to a baking dish and wash 20 minutes x 4 in 1x phosphate buffer with about 700 mls of wash. First two washes without ethidium and last two washes with ethidium.
13. Take picture of gel. Wells should be positive for lots of high molceular weight DNA.
14. Treat remainder of gel like a Southern.

15. Depurinate with 0.25 M HCl (10 cc concentrated to 500 cc water) x 20 minutes. Do not let this go longer.
16. Rinse gel and then denature with 1.5 M NaCl and 0.5M NaOH for 1 hour with gentle shaking.
17. Neutralize with 1M Tris pH 8.0 and 1.5M NaCl.
18. Blot to genescreen with 10X SSC.
19. After transferring, soak blot for 30 seconds in 0.4M NaOH to ensure full denaturation of DNA and then neutralize with 2X SSC and 0.2M Tris pH 8.0 for a few minutes. Dry and then bake in vacuum oven for at least two hours.
20. Prehybridize for at least 4 hours at 72° C. with

5 xSSC	12.5 cc of 20X SSC
5X Denhardt's	5 cc of 50X
1% SDS	2.5 cc of 20%
200 ug/ml of SS DNA	200 ul of 10 mg/ml
water	28 cc

   (50% formamide for 42°C. hybridization)
21. Add radioactive probe and hybridize overnight.
22. Wash at 70° C. with
   1X SSC for 1 hour / 0.5 X SSC for 1 hour / 0.2 X SSC for 1 hour