

PROTOCOL- Isolation of DNA for Mini-Southern Blots From 96-Well Plates

This is Karen Janowski's protocol (University of Alabama)

1. When the majority of clones are fully confluent (culture medium goes yellow within 24 hours of a medium change), aspirate the medium from the 96-well duplicate plates. Wash the plates twice with DPBS. After aspirating the second wash, secure the lid to each plate using tape (do not use Parafilm[®]) and place the plates at -70°C for at least three hours before moving to step 2. Freezing the dishes “cracks” the cells, aiding the lysis and subsequent proteinase digestion and assuring complete digestion of the isolated DNA by the restriction endonuclease(s) used in the analysis.
2. Remove the plates from the freezer and allow them to warm to room temperature for five minutes. Using a multichannel pipettor, add 50 µl of Mini-Southern Lysis Buffer (10 mM Tris pH 7.5, 10 mM EDTA pH 8.0, 10 mM NaCl, 0.5% Sarcosyl, and 1 mg/ml Proteinase K) per well.
3. Incubate the plates overnight at 60°C in a humidified chamber, such as a plastic food container (with a lid) with a wet sponge or dampened paper towels on the inside. Alternatively, place the plates on dampened paper towels and heat-seal in a Kapak[®] pouch, followed by overnight incubation at 60°C.
4. The next day, remove the plates from the incubator. In a low-speed tabletop centrifuge equipped with a swinging-bucket rotor and microtiter plate carriers, briefly spin the plates to rid the lids of condensation.
5. Using the multichannel pipettor, add 100 µl of 75 mM NaCl in cold absolute ethanol (150 µl of 5M NaCl per 10 ml of cold absolute ethanol) per well. Allow the plate to rest on the bench at room temperature for 2 hours or until the precipitated DNA is clearly visible under low-power magnification. The DNA will adhere to the polystyrene dish, so look at the perimeter of each well to see the precipitated DNA. It will look like a spider web.
6. Gently invert the plates into a waste container and allow the ethanol to drain from the wells (the DNA will remain adhered to the plate). Press the plate onto paper towels. Using the multichannel pipettor, add 70% ethanol to wash each well. The use of a squirt bottle is not recommended since a strong stream could detach the DNA from the plate.
7. Invert and press onto paper towels and repeat the wash 2-3 times.
8. After the final wash, invert and press onto paper towels, then allow the plates to air-dry uncovered for 10-15 minutes. It is essential that all of the ethanol dries, or the DNA will not cut. At this point, plates can be stored in a humidified chamber at 4°C until ready to restriction digest.
9. Prepare the Restriction Digest Cocktail (1X restriction buffer specified for the enzyme being used, 100 µg/ml bovine serum albumin, 50 µg/ml RNase, and 10-15 units of enzyme). Prepare enough for n samples (where $n = \text{No. of clones} + 10$) at 40 µl cocktail/sample.

10. Using the multichannel pipettor, add 35 μ l of restriction digest cocktail to each well and mix by pipetting up and down. **Change pipette tips between one row and the next.**
11. Once the cocktail has been added to all the wells, incubate the plates overnight at 37°C (or at the temperature specified for the restriction enzyme being used) in a humidified chamber.
12. The next day, prepare agarose gels (0.8% in TAE) for the electrophoresis of the samples. We use Thermo Scientific Owl A3-1 Wide Gel System. This will accommodate 4 rows of combs, 25 wells each, which is enough for an entire 96-well plate and one molecular weight marker per row. Cast gels thick enough to provide a loading capacity of 40 μ l per well.
13. Remove the 96-well digest plates from the incubator and briefly spin to rid the lids of condensation. Add 5 μ l of loading buffer to each sample and load the gel with entire sample. Electrophorese overnight at 30-35V. When deciding how far to allow the samples to migrate, take into account the size of the fragment(s) being distinguished. The gel can be run further as long as the bands from one row of samples being detected with a probe do not overlap the bands from the lower rows of samples.
14. After photography, perform Southern transfer using your preferred protocol.

