Genomic Southern Protocol

6/27/2002 Leslie Vosshall

1) Cut 10 fly equivalents of DNA (or 10 ug of mouse/rat/human genomic DNA) with 5 ul appropriate enzyme in 100 microliter total volume, overnight.
2) Ethanol precipitate digest (1/10 volume 3M NaOAc pH 5.2, 2.5 volumes absolute ethanol), -20 degrees C for 30 minutes. Spin down DNA, 30 minutes, 15,000 rpm, 4 degrees C. Carefully remove supernatant, add 1 ml 70% ethanol. Vortex 10 seconds. Spin 5 minutes, 15,000 rpm. Carefully remove supernatant, dry down pellet in speedvac briefly. Do not overdry!
3) Resuspend DNA in 30 or 40 microliters TE (10mM Tris HCl pH 7.5-8.0, 1mM EDTA), depending on whether you will run a 15 or 20 well gel. If using Owl 15 well comb=55ul/well final, If using Owl 20 well comb=40ul/well final volume. Make sure that DNA is completely resuspended by gently pipeting up and down and heating sample at 55 degrees C and then gently mixing. The pellet from ethanol precipitation should be completely dissolved.
4) Pour a 0.8% agarose gel, casting it with a single 15 or 20 well comb. Plan on having one marker lane on each end of the gel with your samples in between. Pour a sufficient number of gels to accommodate all your samples. Depending on size of bands expected, load either 1KB Plus ladder and/or lambda/HindIII digested DNA as molecular weight marker. Add 5 ul loading dye to sample and heat, 65 degrees C for 5 minutes. Load gel and run slowly, 50 V for 6 hours or 20 mAmps overnight.
5) Take a picture of gel with fluorescent ruler on either side, aligned with zero at wells, so you can align markers later. Let gel linger under UV illumination on transilluminator for a few minutes to nick DNA (helps transfer of high molecular weight DNAs). Trim gel by cutting off the lower left corner, trim off wells.
6) Acid treat gel 15 minutes at room temperature with 12.5 ml glacial acetic acid/liter of dH2O with agitation.
6) Rinse gel with dH2O and incubate with denaturing solution [0.5N NaOH/1.5M NaCl] with agitation for 30 minutes.
7) Remove denaturing solution, rinse with dH2O, incubate gel in neutralization solution with agitation for 30 minutes to 60 minutes [1.5M NaCl/1M Tris pH 7.5].
8) Remove neutralization solution, incubate gel in 20X SSC for 5 minutes.
9) Set up downward transfer apparatus (Turbo Blotter from Schleicher and Schuell), using 20X SSC as transfer buffer and Hybond N+ as transfer membrane. If you plan to do low stringency hybridization with formamide, use Nitropure (MSI) not Hybond N+ (Amersham). Transfer for 3 hours only!
10) Disassemble transfer and rinse filter with 2XSSC. Mark wells with a black ballpoint pen. UV crosslink DNA to membrane with UV crosslinker. If using Nitropure bake in vacuum oven for 2 hours. Keep filter in 2XSSC until ready to hybridize, at 4 degrees if delay is longer than a few hours.
11) Prehybridize in Buck Buffer (20-40 ml) 1 hour to 6 hours, 65 to 70 degrees C.
(0.5M Sodium Phosphate pH 7.3, 1% BSA, 4% SDS, heated to 65 degrees C if precipitates are present. Add sheared salmon sperm DNA, boiled for 10 minutes, then ice chilled to a final concentration of 0.5 mg/ml)
12) Remove prehybridization solution, denature probe, and add to more Buck Buffer (10-20 ml). Hybridize overnight at 65 to 70 degrees C.(~16 hours).
13) Remove probe and freeze -20 degrees C. Can reuse within one week.
14) Wash 3 X 30 minutes with prewarmed 0.2XSSC/0.5% SDS, 65 to 70 degrees C., with agitation.
15) Remove membrane from wash solution, blot dry on filter paper, tape filter to 3mm paper, orient with fluorescent ink, and cover with saran wrap. Expose to XOMAT-AR X-Ray film in cassette with intensifying screen, -80 degrees C, overnight. Develop film and put up for a longer exposure as necessary.