**DNA DOT BLOTS**

In the slight reverse of most protocols, these DNA dots are used when cold excess DNA is bound to Nitrocellulose filters to pull out specific labeled RNAs or DNAs from a solution or extract.

**Procedure:**

I. Use 1-2 µg DNA/filter

   a. Sonicate in a total vol. of 750 µl water at setting of 2 for 15 seconds -- use vector DNA as negative control.
   b. Add 30 µl of 10 N NaOH chill to denature (final 0.3-0.4 N NaOH)
   c. Dilute 1:1 with 2 M NH₄OAc right before ready to dot. Can make stock of 4 M stock of ammonium acetate, filter sterilized (cannot be autoclaved).
   d. Soak nitrocellulose in 1 M ammonium acetate before applying to manifold. Lay on top of 2 pieces of 3 MM paper, wet with 1 M ammonium acetate. Apply at least 100 µl DNA solution per dot, under very low suction so that solution is drawn slowly through filter.
   e. Wash w/ 0.5 ml 1 M NH₄OAc
   f. Cut filter into strips while still damp. Air dry

Bake at 80°C under vacuum for 2 hours.

Note: if there is any worry about residual DNA on dot blotter, prewash wells with 1 M ammonium acetate prior to applying DNA.