TECHTIPS

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Single Copy Gene Detection Using Two Hour Hybridizations

Detection of single copy genes using Southern blot analysis is often limited by the time required for the hybridization step. Even with the use of reagents that increase the effective concentration of the probe, e.g., dextran sulfate, the hybridization step usually takes 16 hours or longer.' The following TechTip describes the use of Amersham's Rapid Hybridization buffer (RPN1 635/1636) for the detection of a single copy gene in a complex genome employing only a two hour hybridization. The buffer can be stored at room temperature, uses standard probe concentrations and hybridization volumes, and in our hands gives 2-4x more sensitivity than standard overnight hybridizations.

Methods

1. DNA was prepared from chicken embryos using a modified protocol of Wigler et *al.*[4]

2. DNA was digested with *Hind* III, *Bgl* II or *Pst* I using the buffers supplied.

3. Electrophoresis was in 1% agarose gels in Tris-Borate-Na_2 EDTA buffer, pH 8.3. Following electrophoresis, the gel was incubated in the following solutions:

   1.5 M NaCl/0.5 M NaOH for 30 minutes
   1.5 M NaCl/0.5 M Tris pH 7.2 for 30 minutes

4. The gel was blotted to Hybond™ N+ overnight using 20X SSC as transfer solution. The DNA was fixed by wrapping the blot in Saran Wrap™ and exposing to a 301 nm transilluminator for 3 minutes.

5. The blot was pre-hybridized for 15 minutes in 0.2 ml/cm^2 rapid hybridization buffer at 65°C with gentle shaking.

6. The probe, the *Hind* III fragment of plasmid pC1.8, was labeled with 50 µCi [α-^{32}P] dCTP (PB10205, > 3000 Ci/mmol) after excision from a low melting point agarose (FMC) gel using the Megaprime DNA labeling kit (RPN1604-1607).[3,5] The probe had a specific activity of 1.4×10^9 cpm/µg and was purified on a Sephadex G-50 spin column according to Maniatis *et al.*[6] The probe was heat denatured by incubation in a boiling water bath for 5 minutes and quenched the ice. Hybridization was conducted for 2 hours at 65°C in 0.2 ml/cm^2 rapid hybridization buffer with 2 ng/ml probe.

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7. The blot was washed as follows:

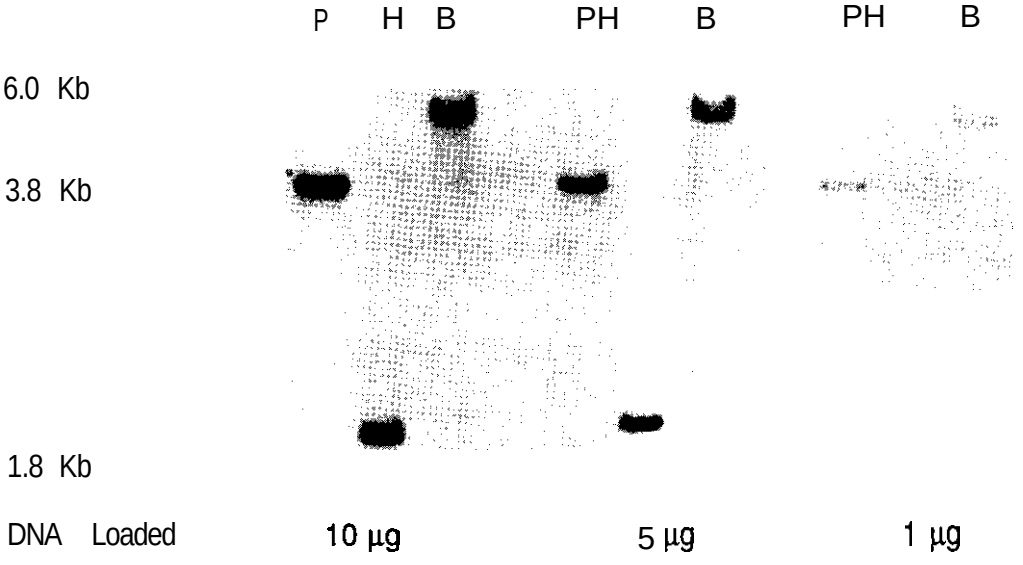
   2X SSC, 0.1% SDS, 10 minutes, room temperature, repeated Ix
   1X SSC, 0.1% SDS, 15 minutes, 65°C
   0.1X SSC, 0.1% SDS, 50 minutes, 65°C (2 buffer changes)

   The stringency of washing may vary with your application, i.e., not all applications require the highest stringency washes.

a. The blot was exposed to Hyperfilm MP (RPN6) for 7 hours at -70°C with Hyperscreens (RPN1662).

Results

Figure 1 displays the detection of the single copy HSP70 gene in chickens: using the protocol described with Amersham's Rapid Hybridization buffer (RPN1635/1636). Within each set of lanes, chicken genomic DNA was cut with either *Pst* I (P), *Bam* HI (B), or ***Hind*** III (H). The amount of DNA loaded in each set of lanes is noted.



References

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