TECH tips

TechTip 115
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Library Screening Using Rapid Hybridizations

Introduction

Screening for recombinant molecules in libraries is a time-consuming process. Using Rapid-hyb buffer (RPN1636) from Amersham™, we have demonstrated reduced-time hybridizations in screening a λgt10 cDNA library. A ten minute hybridization with a ^{32}P random prime labeled probe was used to detect a cDNA clone (see figure 1) which required a 27 hour exposure to film. An even faster method of screening was demonstrated by using a 2.5 hour Rapid-hyb incubation with only a one hour film exposure to detect positives in a plant cDNA library (see figure 2). Hybridization screening in **one day** is possible! Rapid-hyb screening of libraries takes advantage of large amounts of target DNA on colony or plaque lifts. This technique eliminates overnight hybridizations with no reduction of signal to noise ratios. Other Rapid-hyb buffer benefits include the ability to store the buffer at room temperature and the use of standard probe concentrations and hybridization volumes. Protocols for using oligo and RNA probes are also provided. Acceptable hybridization times may need to be determined for partially homologous probes. High sensitivity applications (single copy genomic) require only a two hour hybridization.[1] One hour hybridization is sufficient for most other applications.

Methods

1. Cytoplasmic RNA was prepared from ~10^8 heat shocked (42°C-60 minutes) HeLa cells.[2]

2. Poly $(A)^+$ RNA was isolated using oligo (dT) cellulose chromatography.[3]

3. cDNA was prepared from 5µg poly $(A)^+$ using the Amersham cDNA system (RPN1256).[4]

4. One microgram of cDNA was used to prepare a library using the Amersham λgt10 cloning system (RPN1762). The cloning efficiency was 2.5 x 10^6 pfu/µg cDNA.

5. The primary screen was done by plating the phage on NM514 (selective host) and plaque lifting to Hybond™ N (RPN87N). Plaque lifts were denatured on thick filter paper soaked with 1.5M NaCl, 0.5M NaOH for five minutes then neutralized on filter paper soaked with 1.5M NaCl, 0.5M Tris HCl (pH 7.5) for three minutes plus three minutes on a fresh filter and neutralizing solution. Lifts were briefly washed in 2x SSC, air dried and UV crosslinked, DNA side towards light, using an Amersham UV crosslinker (RPN2501).[5]

6. Prehybridization was for 20 minutes at 65°C in heat-sealed bags with 0.08 ml/cm^2 Rapid-hyb buffer. Hybridization was for 10 minutes at 65°C with the same Rapid-hyb buffer containing 2ng/ml ^{32}P random primer labeled probe using the Amersham Multiprime kit (RPN1601).[6] The probe used for the primary screen was the 2.6 Kb *Hind* III/*Bam* HI fragment of plasmid pH-HSP70 which contains the entire coding sequence of the human HSP70 gene.[7]

7. Membranes were washed as follows.

   a. 2x SSC, 0.1% SDS, room temperature, 10 minutes, repeated one time.

   b. 1x SSC, 0.1% SDS, 65°C, 15 minutes

   c. 0.1x SSC, 0.1% SDS, 65°C, 15 minutes, repeated one time.

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Results

Figure 1 shows a positive from the primary screen of the heat shock library. Figure 2 shows detection of positive plaques in a λgt10 cDNA library after a 2.5 hour hybridization plus *1 hour* autoradiography. The probe was screening for group 2 LEA (Late Embryogenesis Abundant) clones from plants.

Figure 1

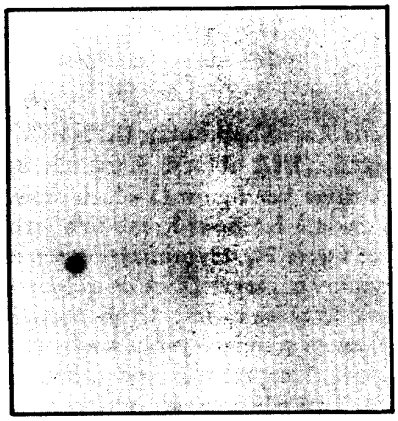

Figure 1. *HSP70 primary screen. Exposed 27 hours to Hyperfilm™ MP (RPN1677) at -70°C with Hyperscreens™ (RPN1669). Probe specific activity was 5.5 x 10^8 dpm/µg.*

Figure 2

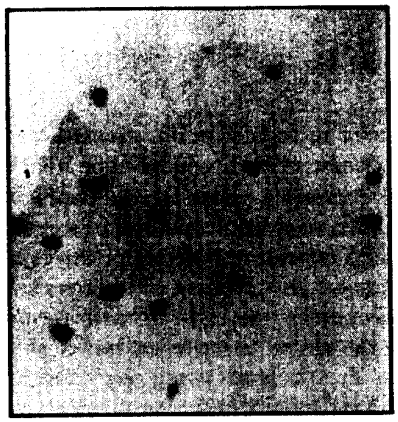

Figure 2. *Quaternary screen. Exposed 1 hour at -70°C with one intensifying screen. Data kindly supplied by Mark Whitsitt (Dr. J.E. Mullet Lab, Dept. of Biochemistry), Texas A&M University.*

References

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