TechTip 130
R922082

Rapid Hybridization Buffer
Time Savings in Hybridizations

Amersham Biosciences has developed a hybridization buffer which allows ultra sensitive detection with hybridizations of 2 hours or less. The Amersham rapid hybridization buffe(RPN1635 [125 ml] or RPN1636 [500 ml]) is designed for use with radiolabeled random prime, RNA or oligonucleotide probes. Single copy genes can be detected with 1-2 hour hybridizations.' Hybridizations using oligo probes take 10-60 minutes. The data presented here, demonstrates a Northern blot rapid hybridization compared to a traditional overnight hybridization. The rapid hybridization saved 24 hours in time and also gave several fold higher sensitivity with the convenience of a pre-made, ready to use hybridization buffer which can be stored at room temperature. Standard probe concentrations and buffer volumes are used with the rapid hybridization buffer.

' RPN1635 and RPN1636 represent an improved version over the previous rapid hybridization buffer, RPN1518 which has been deleted.

Methods

1. Cytoplasmic RNA was prepared from cell line 293 cultures.'

2. Duplicate RNA samples were electrophoresed through a 1.2% MOPS/formaldehyde agarose gel.[3] Gel was washed for 30 minutes in 1 OX SSC (3 changes) and capillary blotted overnight to Hybond N+ (RPN2020B) using 20X SSC as transfer buffer. After blotting, the membrane was cut in two and fixed by baking 1.5 hours at 80°C without vacuum.

3. One blot was pre-hybridized for 15 minutes at 65°C in 0.25 ml/cm^2 rapid hybridization buffer. The conventional blot was pre-hybridized for 1 hour at 42°C in 0.25 ml/cm^2 5X SSC, 5X Denhardt's, 0.1% SDS, 50% formamide, 10% dextran sulfate, 100 µg/ml denatured salmon sperm DNA.

4. The rapid hybridization blot was hybridized for 2 hours at 65°C in 0.25 ml/cm^2 buffer. The control blot was hybridized for 16 hours at 42°C in 0.25 ml/cm^2 buffer. Both blots were hybridized with 2 ng/ml heat denatured pH-HSP70 insert DNA probe. The probe was labeled to 2X10^9 dpm/µg using [α-^{32}P] dCTP (PB10205) and the Megaprime random prime labeling system (RPN1605).

5. Following hybridization, both blots were washed as follows:

   a. 2X SSC, 0.1% SDS, roorn temperature, 2X, 10 minutes each
   b. 1X SSC. 0.1% SDS, 65°C, 1X, 15 minutes
   c. 0.1X SSC, 0.1% SDS, 65°C, 2X, 10 minutes each

   Following washes, the blots were wrapped in Saran Wrap and exposed to Hyperfilm-MP (RPN1677) at -70°C with Hyperscreens (HPN1669).

Results

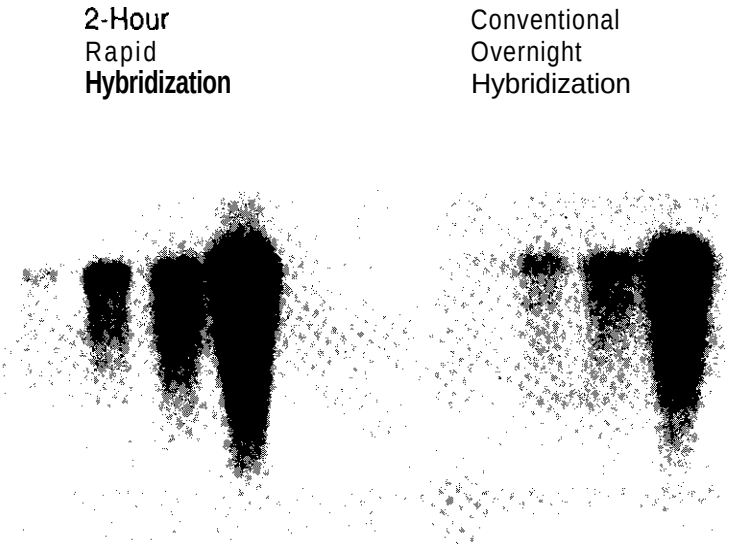

Figure 1. Northern blots (5, 1, 0.5, 0.1 µg each) comparing rapid hybridization buffer to a conventional overnight hybridization buffer. The pH-HSP70 probe recognizes a 2.6 Kb HSP70 mRNA transcript." Hyperfilm-MP was exposed for 8 hours at -70°C with Hyperscreens.

Discussion

The Amersham rapid hybridization buffer (RPN1635/1636) allows sensitive, rapid hybridizations with DNA, RNA, and oligonucleotide probes. Higher target applications (colony, plaque, PCR amplified targets) may need only 10 minutes hybridization to detect target nucleic acid.[5] The benefits of this pre-made, ready lo use rapid hybridization buffer over traditional overnight hybridization buffers include a substantial savings in time (up to 24 hours), superior signal to noise, greater sensitivity, and stability at room temperature. In combination with a rapid random primer labeling system, such as Megaprime (RPN1604-7) which labels probes at high efficiency in only 5 minutes, it is possible to obtain results very quickly with greater sensitivity.

References

1. TechTip 109. Single Copy Gene Detection Using Two Hour Hybridizations. Amersham Biosciences.

2. Graham, F., Smiley, J., Russell, W. and Nairn, R. (1986) J. Gen. Virol., 36, pp. 59-72.

3. Davis, L.G., Dibner, M.D., Battey, J.F., (1986) Basic Methods in Molecular Biology. Elsevier Science Publishing Co. Inc.. New York.

4. Wu, B., Hunt, C., Morimoto, R., (1985) Molecular and Cellular Biology, Vol. 5, No. 2, pp. 330-341.

5. TechTip 115. Library Screening Using 10 Minute Hybridizations. Amersham Biosciences