TECHTIPS

TechTip 134
R932149

Very Rapid Colony and Plaque Screening Using ECL™ Direct DNA Labeling and Detection Systems (RPN3000/3001)

ECL gene detection systems provide many advantages for library screening and other high target applications over radioactive or colorimetric detection [1,2] The technique described here can be completed in less than 2 hours form colony/plaque lift to positive result, compared to 2 days with ^{32}P. Probe labeling takes 20 minutes, hybridization can be achieved in 20 minutes and detection with ECL reagents with exposure to autoradiography film in 5-l 5 minutes (using Hyperfilm™ ECL).

A development of this type greatly increases the speed by which cloning procedures can be carried out as it enables screening and sub-cloning to be achieved in the same working day.

Methods

Colonies and plaques were plated out and lifts taken using positively charged Hybond-N+ (RPN.82B) according to established methods[3,4]. Prehybridization was performed in ECL Gold buffer provided with the ECL Direct kit for 20 minutes during which time the probe was labeled (exactly according to the protocol). Probe was added directly to the prehybridization solution to a concentration of 10ng/ml and hybridized for 20-60 minutes at 42°C. The filters were then washed twice in primary buffer (6M urea, 0.5xSSC, 0.4% SDS) at 42°C and twice in secondary buffer (2xSSC). After draining excess wash buffer, detection was carried out by adding an equal volume mixture of ECL detection reagents directly to the filters to 0.125ml/cm^2. After 1 minute, excess reagent was drained, the membranes wrapped in Saranwrap™ and exposed to Hyperfilm™ ECL (RPN.2 103), typically for 10 minutes Colonies for plaques showing positive signal were then selected for further analysis by other methods such as sub-cloning, DNA sequencing, restriction mapping and PCR.

Summary

Rapid accurate library screening can now be achieved in less than 2 hours using the ECL Direct Systan with the added benefits of safety, speed and probe stability. Labeled probe is stable for at least 6 months if stored in 50% glycerol at -20°C. The ECL Direct system also allows for immediate reprobing without the need for probe stripping. The signal from hybridized probe cannot be re-detected, enabling the researcher to screen the same filters with up to three or four different probes in the same working day.

References

1. Stone, T. and Durrant, I., (1991), Gene Analysis Techniques, 8, pp 230-237

2. Fowler, S.J., Harding, E.R. and Evans, M.R., (1990), Technique-A Journal of Methods in Cell and Molecular Biology, Vol. 2, 5, pp 261-267.

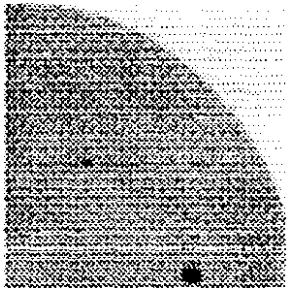
3. Membrane Transfer and Detection Methods, P1/162/86/8, Amersham International PLC, Amersham U

4. Maniatis, T., Fritsch, E.F. and Sambrook, I., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, New York.

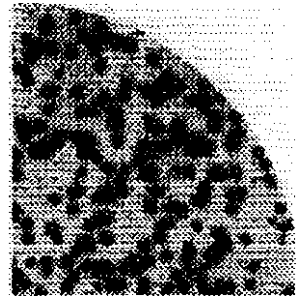
Results

In all cases studied, colonies or plaques corresponding to the current gene of interest were identified. The longest elapsed time from lift to result was 2 hours. The following figures show results from four librarie

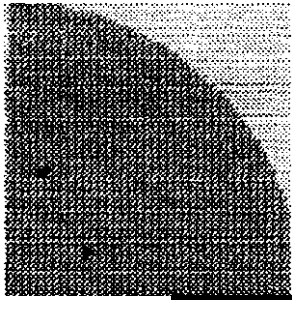
a) Human muscle cDNA library in λgt 11, actin DNA probe. Primary screen 30 min hyb., 20 min exposure



(b) Positive plaque picked from (a) and replated Second screen with actin DNA probe, 10 min hyb., 10 min exposure



c) Human retinal blastoma cDNA library in λCharonBS kindly supplied by Dr. A. Swaroop and Dr. H. Pawar of University of Michigan. *Bst* I fragment 321 probe, 20 min hyb., 10 min exposure



(d) Human heat shock gene HSP70 in pET plasmid HSP70 DNA probe,20 min hyb., 10 rnin exposure