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A simple subtractive hybridization technique employing photoactivatable biotin and phenol extraction

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We report a simple method for nucleic acid subtractive hybridization, that involves hybridization of untreated and photobiotinylated DNA or RNA. After treatment with streptavidin and phenol, hybrids and single-stranded biotinylated material are sequestered to the aqueous/ organic interface while unhybridized material remains in the aqueous supernatant. This procedure is simpler than published methods using either hydroxyapatite (HAP) chromatography (eg 1) or cupric-iminodiacetic acid agarose chromatography of biotinylated nucleic acid (2). The protocol given here is for subtraction of cDNA with RNA, but any combination of nucleic acid may be used.

1. RNA BIOTINYLATION. 10- 30ug poly-A+ RNA (10ul) was mixed with 50ug (50ul) photoactivatible biotin (PAB) (3) (Clontech), and irradiated on ice with a sunlamp for 15 min, according to the manufacturer. Unreacted PAB was removed by making the reaction 100mM Tris pH9.0 and extracting with TE-saturated 2-butanol. RNA was chloroform extracted and ethanol precipitated. Two cycles of photobiotinylation were used to increase density of biotin residues.

2. cDNA/PAB-RNA HYBRIDIZATION. Single-stranded cDNA and PAB-RNA were hybridized at a mass excess of >10-fold PAB-RNA to cDNA, in 50mM HEPES pH7.6, 0.2% SDS, 2mM EDTA, 500mM NaCl (HB). NaCl was added last to aid nucleic acid solubility. The reaction (volume 3 - 5 ul in a 0.5ml Eppendorf tube) was overlaid with paraffin oil and heated to 95°C, 2 min. Hybridization was at 65°C, until appropriate Cot was reached- typically 24 - 48 hours.

3. SEPARATION OF HYBRIDS FROM SINGLE-STRANDED DNA. (A) The hybridization reaction was transferred to 100ul HB without SDS (HB-SDS). 5ug Streptavidin (BRL) was added and the mix incubated at room temperature for 1 min. The reaction was extracted with TE- saturated phenol:chloroform (1:1) (P/C). The aqueous phase, containing unhybridized cDNA, was removed, and the interface and organic phase containing cDNA/PAB RNA hybrids was washed with 25ul HB-SDS. This streptavidin/ P/C treatment was repeated on pooled aqueous phases twice more. Aqueous phases were chlororform extracted and ethanol precipitated. A second similar subtraction could now be performed. **(B)** The procedure described was also used to obtain positively selected cDNA (ie cDNA hybridizing to a particular RNA population). After each P/C extraction, the interface was transferred to TE buffer. The mixture was heated to 95°C for 10 minutes to denature the hybrids and streptavidin/ P/C extracted. The aqueous phase contained released cDNA.

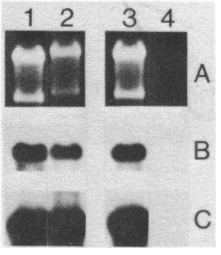

FIGURE 1 Total RNA from mouse tissue culture cells was mock photobiotinylated (1, 3), or photobiotinylated (2, 4). RNAs were ethanol precipitated (1, 2) or treated with two streptavidin/P/C cycles and precipitated (3, 4). Equal volumes of precipitates were run on agarose gels and stained with ethidium bromide (A), or analysed by Northern hybridization for *B*-actin RNA (B-10 hour-, C-4 day exposure).

4. DISCUSSION. Fig. 1 shows that PAB-RNA was very efficiently removed by streptavidin/ P/C treatment. Phenol and streptavidin were both required for maximal removal of PAB-RNA, as were monovalent cations- 500mM NaCl was optimal (data not shown). Efficiencies of subtractions employing biotin/ phenol and HAP were essentially the same when performed in parallel. This protocol was used to construct subtracted cDNA libraries enriched for RNAs specifically expressed in the head of Xenopus laevis embryos and for sequences differentially expressed between mouse lymphoid cell lines (data available on request).

(1) Davis, M. et al (1984) Proc. Natl. Acad.Sci.USA 81,2194-2198.(2) Welcher, A. et al (1986) NAR 14,10027-10044. (3) Forster, A. et al (1985) NAR 13,745-761. Supported by NIH grant CA42571 to TSJ and ACS fellowship PF-02714-03 to HLS. HLS thanks Hal Weintraub for support.