A simple and efficient cDNA library subtraction procedure: isolation of human retina-specific cDNA clones

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The characterization of several recently isolated genes encoding components of the photo-transduction cascade has provided candidate genes for human and mouse retinal degeneration disorders (1). We have used a biotin-based subtraction procedure (see figure) to enrich human adult retina cDNA library for specific clones. The directional cDNA libraries from the human adult retina and JY lymphoblastoid cell line were constructed in Charon BS (-) vector (2) as described (3), and were then transferred to plasmid BS (-) (2). The plasmid retina cDNA library was used to generate single stranded (ss) circular DNA with helper phage R408 (Stratagene protocol). The double stranded plasmid DNA from JY cDNA library was used to synthesize biotinylated run-off transcripts with Bio-11-UTP (Enzo Biochem). Since the two cDNA libraries have directional cDNA inserts, T3 RNA polymerase would generate JY biotinylated RNA that is complementary to the orientation of cDNAs in retina ss DNA species. Typically, 0.2–1 µg of ss DNA from retina library (corresponding to about 2×10^5 – 10^6 amp-resistant colonies) was annealed to 20–40 µg of biotinylated RNA from JY library in a sealed capillary at 65°C for 16 hr. The reaction contained 0.4–0.5 M sodium phosphate pH 7.2, 10 mM EDTA and 0.1 % SDS in 20–25 µl volume. After hybridization, the mixture was incubated with 250 mg vectrex-avidin (Vector laboratories) [presaturated with binding buffer (100 mM Tris.Cl pH 7.5, 150 mM NaCl) containing 50 µg/ml denatured salmon sperm DNA and then washed 3 times with binding buffer alone] in two batches for 30 min to 2 hr at 22°C. The ss DNA in the unbound fraction (enriched for retina specific ss DNA) was precipitated in the presence of glycogen, and an aliquot of it was used directly to electrotransform XL1 blue cells (Bio-Rad Gene Pulser/Pulse Controller). Control experiment did not include any biotinylated RNA. Between 2–15% of the clones are recovered after subtraction. The ratio of blue versus white colonies is increased dramatically in the subtracted library because of the simultaneous enrichment of non-insert containing ss DNA molecules. Forty randomly picked white colonies from two subtracted retina libraries were characterized by Southern analysis of DNA from cDNA libraries, and/or by Northern analysis of total RNA. Almost 70% of the clones are not detectable in the JY cDNA library against which the retina library was subtracted (data not shown), and this included several novel retina-specific cDNAs. Similar results were obtained with the analysis of 15 cDNA clones from a subtracted retinal pigment epithelium library. While this work was in progress (4), several reports (5–7) described

methods using photobiotinylated ss DNA species. However, the complementarity of the hybridizing species from two directional cDNA libraries, use of RNA polymerase to obtain large amounts of biotinylated driver RNA and direct transformation of ss DNA obtained in the unbound fraction further improves the efficiency of subtraction procedure described here.

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