Preparation of Magnetic Oligo(dT) Particles

Methods for mRNA extraction using oligo(dT)-coupled magnetic beads have been described previously (1), and mRNA extraction kits based on this technique are commercially available. Unfortunately, the price of oligo(dT)-coupled beads makes this technology too expensive for routine mRNA extraction. Here we describe an efficient procedure for covalent coupling of oligo(dT) to magnetic particles.

Poly(dT) oligonucleotides are linked in a simple one-step reaction, adapted from Rasmussen et al. (2), by means of a 5′-amino linker to carboxyl-coated magnetic particles. The resulting oligo(dT)-coupled magnetic beads are of high quality and have been used successfully for mRNA extraction and as primers for cDNA synthesis. Since the whole coupling procedure can be performed within half a day, employing only standard laboratory equipment and inexpensive starting material, the manufacturing costs of these beads are much below the price of commercially available oligo(dT)-coupled magnetic beads.

The material required is as follows: 5′-amino modified oligo(dT)₁₃₀, 1-methylimidazole; 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC); and carboxyl-terminated magnetic beads (e.g., Advanced Magnetics, Cambridge, MA, USA, or Sigma Chemical, St. Louis, MO, USA).

The carboxyl-terminated magnetic beads are washed once in 100 mM imidazole buffer, pH 7.0. After collecting the beads with a strong magnet (not by centrifugation!), the supernatant is aspirated. One milligram of beads is incubated with 20 µg of 5′-amino-oligo(dT)₁₃₀ in 0.6 mL of freshly prepared coupling buffer (100 mM EDC in 100 mM imidazole buffer, pH 7.0) for 3 h at 50°C. Because the reproducibility of the coupling reaction depends mainly on the activity of EDC, EDC has to be stored desiccated at 4°C, and the coupling buffer has to be prepared fresh immediately before use. To prevent the beads from sedimenting, the tube should be rotated or inverted continuously. We routinely prepare 80 mg of beads with 1.6 mg 5′-amino-oligo(dT)₁₃₀ in 50 mL of coupling buffer in a 50-ml tube.

After incubation, the beads are collected with a magnet, the supernatant is aspirated, and the beads are washed three times with 2× standard saline citrate (SSC) containing 0.5% sodium deoxycholate (SDS) at room temperature and twice with RNase-free water at 65°C. The beads are resuspended in phosphate-buffered saline (PBS) containing 0.2% NaNO₃ and stored in aliquots at a concentration of 5 mg/mL at 4°C.

The binding capacity of the oligo(dT) beads can be determined easily by extracting RNA with decreasing amounts of beads in parallel tubes and measuring the amount of eluted RNA by wavelength scanning from 220–300 nm or by Northern blotting. In Figure 1, 80 mg of heart tissue were extracted with 10–250 µg of oligo(dT) beads in parallel samples. In this example, 100 µg of oligo(dT) beads were sufficient to extract poly(A)⁺ RNA completely, since 250 µg of oligo(dT) beads increased only the unspecific background, not the specific signal. Following this protocol, a binding capacity of greater than 20 µg of bound poly(A)⁺ RNA per mg beads is reproducibly determined.

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Figure 1. Poly(A)⁺ RNA was extracted from 80 mg heart tissue with the indicated amounts of oligo(dT) beads as described in Reference 1. The RNA was glyoxylated, separated in a 1.2% agarose gel and transferred to a positively charged nylon membrane (Boehringer Mannheim, Mannheim, Germany). After hybridization with a digoxigenin (DIG)-labeled cRNA probe, nerve growth factor (NGF) mRNA was detected by CSPD⁺ chemiluminescence (Tropix, Bedford, MA, USA). Hybridization and detection were performed under standard conditions as described in the Boehringer Mannheim DIG-detection protocol.
achieved. After each extraction, the beads should be washed twice with RNase-free water and stored in PBS containing 0.2% NaN₃. The beads can be reused at least 20 times.

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Isolation of 3.5-kb Fragments on Magnetic Solid Supports

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Technical improvements in solid-phase sequencing of polymerase chain reaction (PCR)-amplified DNA have recently been made in several areas, including clinical microbiology, gene sequencing and genetic diagnostics. Magnetic beads are quickly becoming the solid support medium of choice for most nucleic acid purifications. However, attempts to bind DNA fragments larger than 700 bp by previously established protocols led to sharp decreases in binding efficiencies. We have developed a simple method to effectively bind 5‘ biotinylated 3.5-kb PCR fragments (1,2) to streptavidin-coated Magnetic Porous Glass® (MPG) particles (CPG, Lincoln Park, NJ, USA) as a means for automated fluorescence sequencing.

RNA was extracted from fresh patient blood samples using Trizol® Reagent (Life Technologies, Gaithersburg, MD, USA), and the expressed target of interest was reverse-transcribed into cDNA. This cDNA was then amplified in a primary Long PCR (3) reaction (PCR 1). One hundred-microliter

![Figure 1. MPG-immobilized 3.5-kb PCR fragment sequenced using the AutoRead™ Sequencing Kit and the ALF™ DNA Sequencer. Run shows clean peak resolution over 710 bases from the sequencing primer.](image-url)