3.7. Size Fractionation

There are many types of filtration media used to separate DNA molecules. Sephacryl S-500 medium separates efficiently in the 2-kb size range. Drip columns made with Sephacryl S-500 medium separate by size, the larger cDNA molecules eluting from the column first and the small unligated adaptors and unincorporated nucleotides eluting later. The cDNA will not have a high number of counts, but will be detectable by a handheld monitor at ≤250 cps.

3.7.1. Drip-Column Preparation

1. Discard the plunger from a 1-mL plastic syringe and insert a small cotton plug. Push the cotton to the bottom of the syringe.
2. Fill the syringe to the top with Sephacryl S-500 filtration medium.
3. Place the syringe in a rack and allow the column to drip "dry."
4. Fill the syringe up to ~0.5 cm from the top with medium, and drip through as in **step 3**.
5. Rinse the column with two aliquots of 300 L of 1X STE buffer (total wash volume of 1200 μL). Drip-dry after each addition of buffer.

3.7.2. Collecting Fractions

1. Pipet the cDNA into the washed Sephacryl S-500 drip column, and allow to drip through. This is fraction 1. The recovery volume is ~150 μL and does NOT contain cDNA (*see* **Note 6**).
2. Load two more aliquots of 150 μL of 1X STE buffer on the column and drip through. These are fractions 1 and 3.
3. Collect fraction 4 in fresh tube. Load 150 μL of 1X STE buffer and drip as before.
4. Collect fraction 5 as in **step 3**. Two fractions are usually adequate. The size of the cDNA decreases in each additional fraction. Most of the radioactivity will remain in the column owing to unincorporated nucleotides. Discard the radioactive drip column appropriately.
5. Remove 5 μL from each fraction (or up to 1/10 of the fraction volume) for analysis of cDNA size on a 5% nondenaturing acrylamide gel. These aliquots can be frozen at –20°C.
6. To remove any residual enzyme from previous reactions, phenol-chloroform/chloroform extract (*see* **Note 5**).
7. Add twice the volume of 100% (v/v) ethanol to precipitate the cDNA.
8. Place on ice for 1 h or at –20°C overnight.

3.8. Quantitating the cDNA

1. Microcentrifuge the fractionated cDNA at maximum speed for 30–60 min at 4°C. Carefully transfer the ethanol to another tube and monitor with a Geiger counter. Most of the counts should be present in the pellet. Discard the ethanol appropriately.