Centri-Sep Sample Filtration

PURPOSE: Prior to sample electrophoresis, sequenced products must be purified in order to remove unincorporated dye terminators.

A. Procedure for Single Columns

1. Gently tap columns to insure dry gel material has settled to bottom of spin column. Remove top column cap and add 800 µL of sterile or UltraPure dH_2O to one column for each sequencing reaction.

2. Replace top cap and mix thoroughly by inverting column and vortexing briefly. It is important to hydrate all of the dry gel. Allow columns to hydrate for at least 2 hours at room temperature. As the columns are hydrating you will need to label one sample collection tube (1.5 mL microcentrifuge tube) for each sequencing reaction. You will also need one wash tube for each hydrated column. These do not need to be labeled.

3. Once the columns are hydrated, remove any air bubbles by inverting the column and sharply tapping the column, allowing the gel to slurry to the opposite end of the column. Stand the column upright and allow the gel to settle while in a centrifuge tube rack.

4. Once the gel is settled, remove first the top column cap, and then remove the column end stopper from the bottom. Allow excess column fluid to drain into a wash tube by first gently tapping the column into the wash tube then allowing to sit for approximately 5 minutes. Remove the column from the wash tube, discard the liquid and reinsert the column into the wash tube.

5. Spin the assembly at 700 x g for 2 minutes to remove interstitial fluid. Be sure to note the orientation of the columns. At this point the columns should be used as soon as possible for the loading of cycle-sequenced DNA product.

6. Load entire sequencing reaction volume (20 µL) to the top of the gel. Be careful to dispense sample directly onto the center of the gel bed without disturbing the gel surface.

7. Place column into labeled sample collection tube and spin at 700 x g for 2 minutes maintaining original orientation. The purified sample will collect in the

bottom of the tube.

8. Discard the column and dry the sample in a vacuum centrifuge (approximately 15-20 minutes). Do not over dry samples.

B. Procedure for Centri-Sep 8 Strips

1. Determine how many strips are necessary to filter the amplified samples. Separate the desired number of strips by cutting the foil between the strips with scissors.

2. Open the well outlets on each strip by cutting off the bottom edge with scissors. Cut at the narrowest part of the bottom of the tube.

3. Peel off the top foil and arrange the strips evenly on deep-well centrifuge plates. Spin the plates at 750 rcf for 2 minutes to remove the liquid.

4. Arrange the newly drained strips on a new 96-well plate. Add the amplified sample to each column.

5. Once all of the samples are loaded, place the 96-well plate with the Centri-Sep 8 Strips into the centrifuge, and spin at 750 rcf for 2 minutes.

6. Confirm that all of the samples passed through the strip into the wells of the 96-well plate, and discard the Centri-Sep 8 Strip.

7. Evaporate the samples in the 96-well plate at 75 ^{O}C in a thermalcycler with the lid open.

8. If the samples are not going to be loaded immediately, they should be stored as dried pellets at 4°C for no longer then 14 days. When ready, proceed to 3130xl setup.

CENTRI-SEP SAMPLE FILTRATION		
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