

## **96-well plate purification of sequences**

- a. Add dry Sephadex G-50 superfine (Fisher Cat# 45-000-015 \$375/100g) to the Millipore 45 ul Column Loader (cat.# MACL09645 - \$437). Only fill as many wells as needed to purify reactions, recover excess on clean foil.
- b. Remove the excess of resin from the top of the Column Loader with the scraper supplied.
- c. Place MultiScreen HV Plate (Millipore MAHVN4550 - \$893/50) upside-down on top of the Column Loader.
- d. Invert both MultiScreen HV Plate and Column Loader. and tap on top of the Millipore Column Loader to release the resin.
- e. Using a multi-channel pipettor, add 300 ul of ddH<sub>2</sub>O to each well to swell the resin. Let stand at room temperature for 1-3 hours.
- f. Once the minicolumns are swollen in MultiScreen plates, they can be sealed with saran wrap and stored in the refrigerator at 4 deg C for several days. A batch of plates also can be stored in the refrigerator at 4 deg C for several weeks in a sealed plastic container with a damp towel to assure the plates are kept moist.
6. Spin through excess liquid over a microtiter plate (can reuse this over and over for this purpose) by taping the column plate to the collection plate and centrifuging for 5' at 2000 rpm.
7. While you wait, ADD 20 ul ddH<sub>2</sub>O to each reaction. Align over clean microtiter plate (can see if it fits over sequencing plate). Add reactions to the top of columns without touching them. Spin 5' at 2500 rpm. Should recover approx. 20 ul. Freeze reactions if you cannot sequence that day.
8. Run sequences as they are on the automated sequencer (Applied Biosystems).