Sample Prep Protocols

Following cycle sequencing samples must be processed before running on a sequencer. Purification by size exclusion using G50 gives high quality results.

Equipment Required

Centrifuge-capable of spinning a microtiter plate assembly at 1000g
Low volume multichannel pipettor. We use a Multimek or FX

Supplies required

G50 - we use Sigma G50-50
Microtiter filter plates - Millipore MAHV N45 10 or 50 (10 is for a 10 pk and 50 is for a 50 pk)
Column loader - Millipore MACL 096 45
Extra Scrapers - MACL 0SC 03
Ultrapure DI water
PE MicroAmp Optical 96-Well Reaction Plates - N801-0560

G50 Plate Prep

1. Load dry G50 into wells of microtiter plate using the column loader according to mfg. protocol.
2. Hydrate with 300 ul of DI water/well. We use a Biomek 2000
3. Let stand 3-4 hours before use, do not let the plate dry out. Plates can be stored at 4C for up to 1 week if kept in a humid chamber (Ziploc with wetted paper towel is sufficient). Warm refrigerated plates to RT before use (1-2 hrs).

Sequencing Sample Purification

1. Pack column by centrifuging at 1000g for 5 min. (Don't forget the collection plate).
2. Remove the collection plate (now full of water) and place the G50 plate on the deck of the Multimek.
3. Place the sequenced plate on the appropriate place on the deck of the Multimek.
4. Using the Multimek, load 10ul of sequencing reaction onto the top center (location is very important) of the G50 plate
5. Mark the A1 corner of the MicroAmp plate and place under the G50 plate (check orientation)
6. Centrifuge at 1000g for 5 min. It is critical that the packing spin time and the purification spin time are the same. Use an accurate timer
7. Plate are dried using a speed-vac and stored at -20 (long term storage).
8. Plates are resuspended using the Multimek prior to running on a sequencer.

Cost is approximately $13.00/96-well plate