

Clean-up using Edge Biosystems Spin Columns

Purpose: to use an Edge Biosystems gel filtration plate in order to remove dye terminators, dNTPs, salts and other low molecular weight materials from the sequencing reaction.

Materials:

- Optical 96-Well Reaction Plate (MicroAmp, part # 4306737)
- 30 μ L Matrix pipette tips (Matrix, cat # 7631)
- Matrix 12.5 μ L pipettor (Matrix, cat # 2003)
- Spin Columns (Edge Biosystems, Part # 47938)
- 96-well plate (March Bioproducts, cat # 0800/150)
- 96-well polystyrene U bottom lids (VWR, cat # 62402-929)
- Applied Biosystems 3730 DNA Analyzer and software

Procedure:

- 1- Remove reaction plates from the thermal cycler or from storage in 20 °C freezer.
- 2- Gently vortex and spin down each plate in centrifuge at 200 RCF for 15 seconds.
- 3- Remove the bottom adhesive tape, and then the top adhesive tape from the thawed 96-well spin column plate. Cover with polystyrene lid.
- 4- Stack the 96-well spin column plate on top of a 96-well waste plate. Place this assembly in the centrifuge carrier.
- 5- Centrifuge for 3 minutes according to the centrifuge settings listed in Table #2.

Table #2: Centrifuge settings for spin columns

Parameter	Value
RPM	265
RCF	848
Acceleration	9
Deceleration	10
Radius	108 mm

- 6- Discard eluate.
- 7- Transfer the reaction samples from the reaction plate (volume of 20 μ L) to the center of each well in the spin column plate using a multi-channel pipette (ideally, one should use a 12-channel pipette, so as to be able to differentiate pipetting errors in this step from the “reaction set-up” step where an 8-channel pipette is used to transfer template and add master mix to the reaction plates). Pipet slowly, making sure that the pipette is perpendicular to the plate. Do not touch the sides of the wells. Cover with lid.
- 8- Stack the spin-column plate on top of the previously labeled corresponding optical 96-well reaction plate. Place the assembly in the centrifuge carrier and centrifuge for 5 minutes according to the centrifuge settings listed in Table #2.
- 9- Retain eluate in the optical 96-well reaction plate, making sure that the volumes in each well are uniform. Note any discrepancies. This eluate contains the purified sample ready for loading onto the sequencer. Discard spin column plate.

- 10- Heat-seal the optical 96-well reaction plate.
- 11- Place in sequencing plate holder. Ensure the lid is clasped correctly to the bottom plate holder. Place in “In Stack” of 3730XL sequencer.

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