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Tech Note

Using Millipore's Vacuum Manifold and Montage™ SEQ_{96} Kit on the Precision™ Microplate Pipetting System

Semi-Automation of Sequencing Reaction Cleanup

To generate high quality DNA sequence data, unincorporated dye terminators and salts must be removed from sequencing products prior to both capillary and slab gel electrophoresis. The uniform desalting and recovery capabilities of the Montage SEQ_{96} Sequencing Reaction Cleanup Kit eliminate the capillary-to-capillary variability that is sometimes observed with sequencing reactions prepared by ethanol precipitation.

Here we describe the use of the Precision™ Microplate Pipetting System to semi-automate the steps necessary to carry out the procedures outlined in Millipore's Montage™ SEQ_{96} Sequencing Reaction Cleanup Kit. The procedure uses a Millipore vacuum manifold on the deck of the Precision Microplate Pipetting System with the user turning the system vacuum on and off as needed.

Figure 1. Precision™ Microplate Pipetting System with Populated Deck. The Precision™ Microplate Pipetting System has two boxes of pipette tips located in positions A and D. The Montage™ SEQ_{96} plate and vacuum manifold has been located at position C using a holder (P/N 7112110) designed to accommodate the tubing connector in the front. A separate holder (P/N 6002076) is used to secure a 96-well skirtless PCR plate and then positioned on the deck at position B using the same holder as the vacuum manifold. Injection solution is located in the reagent reservoir at position E, while the injection plate is located at position F.

BioTek Instruments, Inc., P.O. Box 998, Highland Park, Winooski, Vermont 05404-0998 USA
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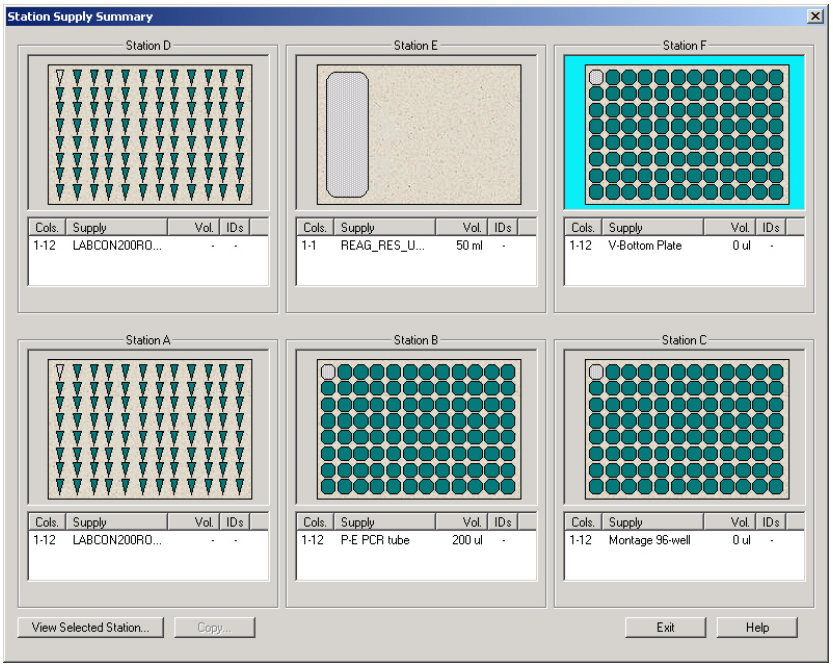

Figure 2. Deck Layout of the Precision™ Microplate Pipetting System. The positions of the necessary hardware on the deck, as described in Figure 1, are defined in the Precision Power™ Software (BioTek Instruments). A graphical representation is then provided within the software.

Procedure

1. Transfer 30 µl of injection solution (Position E) to the sequencing reaction samples plate (Postion B).
2. Transfer 30 µl of the diluted samples (Position B) to the SEQ_{96} plate (Position C). Note that the SEQ_{96} plate is located on top of Millipore vacuum manifold (Millipore P/N SAVM38401). In addition, the vacuum manifold is positioned on the deck using a manifold holder (P/N 7112110).
3. Manually turn on vacuum pump and open valve to the manifold. Manually adjust regulator to a consistent pressure. Note we used 23 – 25 in. Hg. Apply vacuum for 2 – 3 minutes
4. Transfer 30 µl of injection solution (Position E) to the SEQ_{96} plate (Position C).
5. Apply vacuum for 3 – 4 minutes.
6. After all wells are emptied, turn off vacuum and open regulator valve to release any residual vacuum. Regulator should read 0 in. Hg.
7. Transfer 25 µl of injection solution (Position E) to SEQ_{96} plate (Position C).
8. Mix each sample 20 times.
9. Transfer 25 µl to the injection plate (Position F).

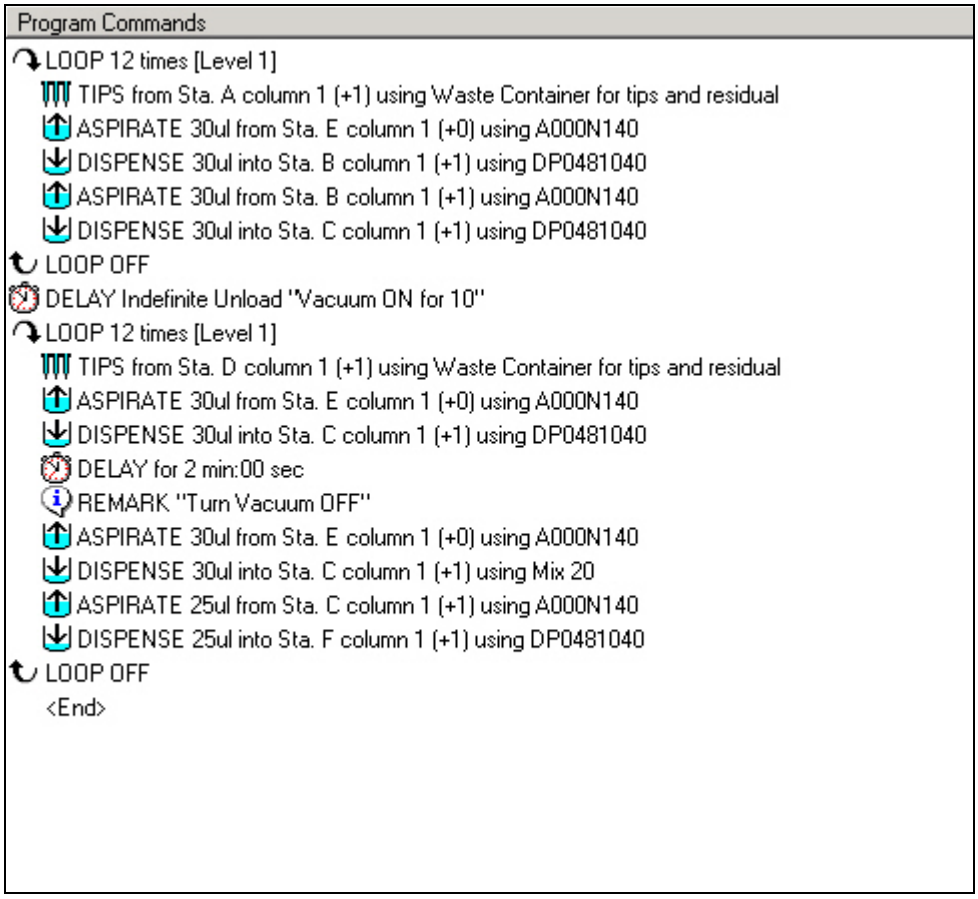

Figure 3. Programming Steps used by the Precision™ Microplate Pipetting System.

Discussion

The Montage™ SEQ_{96} kit can be semi-automated using any of BioTek's Precision Microplate Pipetting Systems. The pipetting systems can be used to dispense reagents and transfer products to and from the Montage SEQ_{96} plates respectively, but control of the vacuum is performed manually. While in this example the vacuum apparatus was positioned on the Precision's deck (Figures 1 and 4), one could also configure the assay to be run with the vacuum manifold at some other location. The user would of course be required to manually transfer the plate to the vacuum. In addition to the manifold, Millipore's vacuum plate apparatus is provided with a four-position connector that links the vacuum pump, gauge, manual vacuum regulator and the vacuum manifold (Figure 5). All of these pieces are connected with self-sealing quick connectors for easy attachment and removal. In order to use the manifold system on the deck, the tubing between the manifold and the 4-way connector was lengthened in order to allow for free movement of the deck.

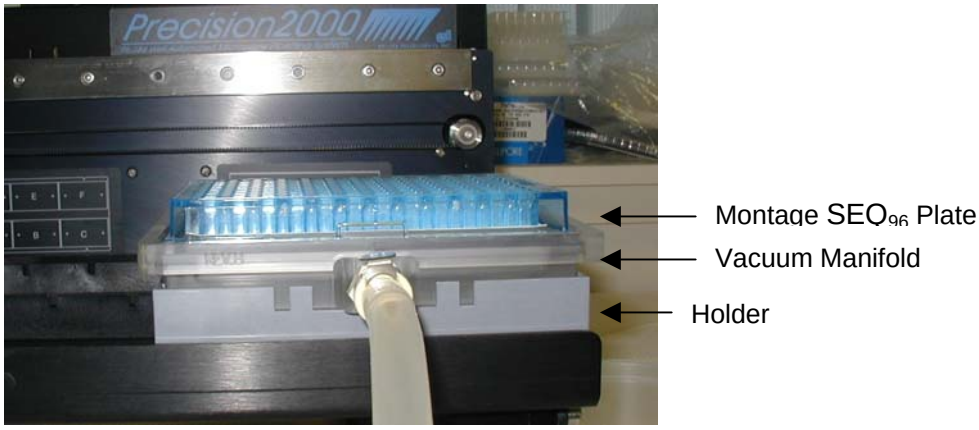

Figure 4. Vacuum manifold and holder with a Montage SEQ$_{96}$ plate. The vacuum manifold is situated on the deck using a multipurpose holder (P/N 7112110), which has been designed among other things to hold the vacuum manifold. The Montage SEQ$_{96}$ plate is situated on the vacuum manifold.

Figure 5. Tubing Connections of Millipore's vacuum apparatus. The pressure regulator, vacuum gauge, shutoff valve and pump are connected using 0.25" inner diameter tubing and quick-connect fittings.

There are a number of different configurations that can be used with these types of plates. However, it is necessary to have the manifold in one of the front positions of the deck to allow the tubing to drape forward. Position C was found to be the most acceptable, as there is considerably less interference with the tubing. In this example we used a skirtless 96-well PCR plate for the sequencing reaction samples. This necessitated the use of BioTek's PCR plate holder to provide the rigidity necessary to keep the plate flat. Many 96-well PCR plates that have side skirts can be used as regular microplates. These types of PCR plates can often be used with the standard Precision furniture, as they do not require special holders to keep them flat.

Programming of the assay process is made very easy with Precision Power™ Software (Figure 2 and 3). The locations of reagents and vessels on the deck are first defined (Figure 2), and then the programming steps to get tips, transfer and mix fluids are created (Figure 3). The programming of, repetitive steps in plate-to-plate transfers can be easily accomplished using Precision Powers flexible loop functions.

Rev. 10/27/06