

Biomek PCR Clean-up

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This procedure removes the PCR reagents from the PCR product, resulting in 100 ul of clean PCR product. The next procedure is Quantitation.

Input: PCR product plate A1

Output: Cleaned-up PCR product plate A2

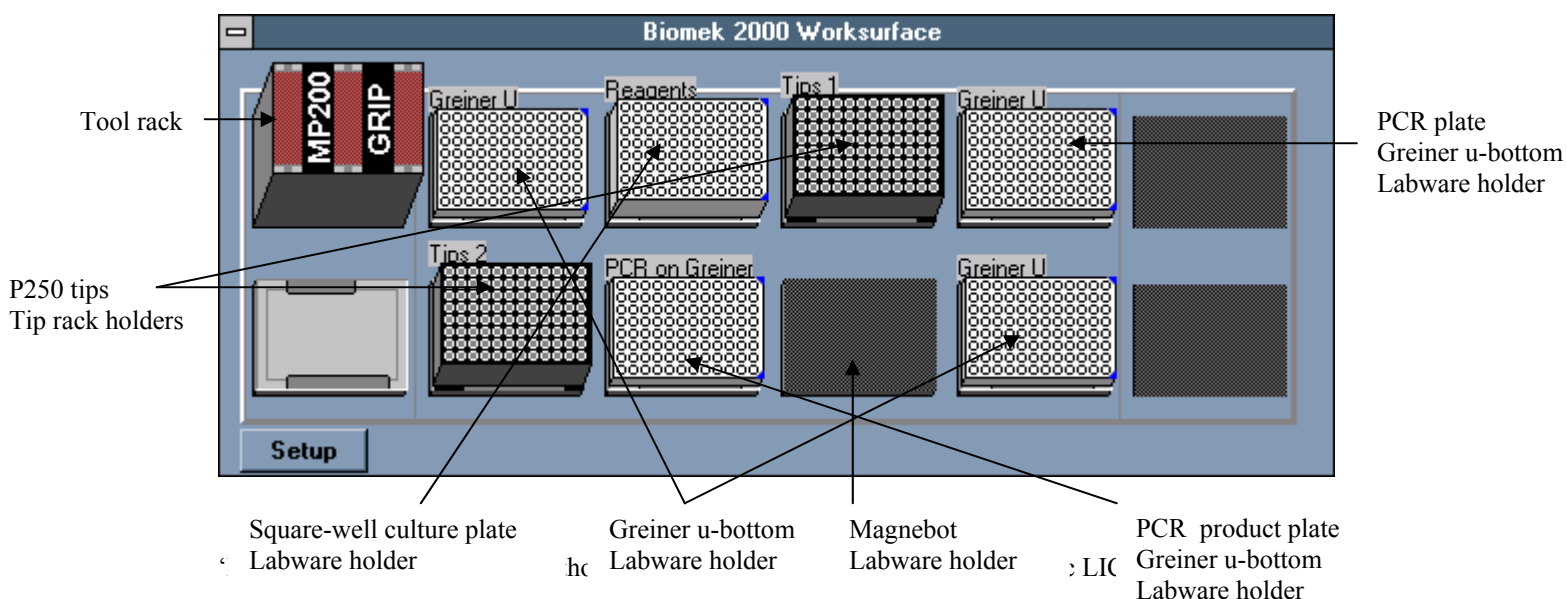
Materials/Reagents/Equipment	Vendor/Location
Reagents	
Wizard MagneSil PCR Clean-Up System	Promega (Cat#:A1930)
PCR product in PCR plate	Freezer
80% ETOH	
Disposables	
Square-well 2ml culture plate	E and K (Cat#: 662000)
PCR plate & mat	E&K (Cat#: 489096 & 402096)
96-well u-bottom plate	Greiner (Cat#: 650101)
Equipment	
Biomek 2000	Beckman Coulter
Magnebot plate	Promega, (Cat# 725226)

Procedure

I. Preparing Reagent plate:

1. Open the yellow Wizard MagnaSil box which contains the PCR clean-up reagents. Shake the MagnaSil YELLOW bottle which contains the magnetic beads, to resuspend them before pipetting.
2. In a square-well 2 ml culture plate, dispense the following reagents:
 - Column 1: MagnaSil YELLOW beads: For each well add 200 ul + (100 ul x # of well in ROW)
 - Column 3: Wash Solution: For each well add 200 ul + (100 ul x # of well in ROW)
 - Column 5: 80% ETOH: For each well add 200 ul + (300 ul x # of well in ROW)
 - Columns 9,10, and 11: 1 ml autoclaved water (for rinsing tips)
 - Column 12: nuclease-free water: For each well add 200 ul + (100 ul x # of well in ROW)

II. Setting up the Biomek worksurface:



- ___1. Place the reagent plate at A3.
- ___2. Place the Magnabot magnet at B4.
- ___3. Place clean u-bottom Greiner plates at A2 and B5.
- ___4. Place support u-bottom Greiner at A5 and B3.
- ___5. Place the PCR reaction product plate at B3.
- ___6. Place a clean PCR plate at A5 for the cleaned-up PCR product.

III. Running Biomek method:

- ___1. Double click on Biomek Lab Book Manager icon on the desktop.
- ___2. Select the **PCR CLEANUP** folder and click on **Set as Current Lab Book**.
- ___3. Click **Close**.
- ___4. Double click on Biomek Edit icon on the desktop.
- ___5. Click **Method → Open → Select PCR Cleanup 081103**.
- ___6. Click on **Edit → Patterns → Select PCR on Greiner → Allow Changes →** Set the pattern to match PCR product plate → Click OK to confirm the pattern.
- ___7. Click on **Edit → Patterns → Select Entire PCR Plate → Allow Changes →** Set the pattern to match the PCR product plate → Click OK to confirm the pattern.
- ___8. Click on **Edit → Patterns → Select Transfer plate → Allow Changes →** Set the pattern to match the PCR product plate → Click OK to confirm the pattern.
- ___9. Check Reset Tip Rack command lines (17 lines) in method to match tip boxes : double-click on Reset line to open dialog box.
- ___9. Click on the running man button to start the method. (Note: Save all the settings and click **Accept All** to confirm the configuration.)

Note: The method takes approximately 45 minutes in addition to the 1 hour air-drying time.

IV. After running the method:

- ___1. Cover cleaned-up PCR product plate with mat and store in Freezer318.
- ___2. Put extra MagneSil Yellow beads back to the MagneSil Yellow Beads container. Store them with other MagneSil reagents in Wizard MagneSil box.
- ___3. Discard Reagent Plate and 2 Greiner u-bottom processing plates.
- ___4. Remove used tips from tip rack.
 - Tip usage: Tips 1 at A4: # of columns x 1
 - Tips 2 at B2: # of columns x 2