Biomek Normalization

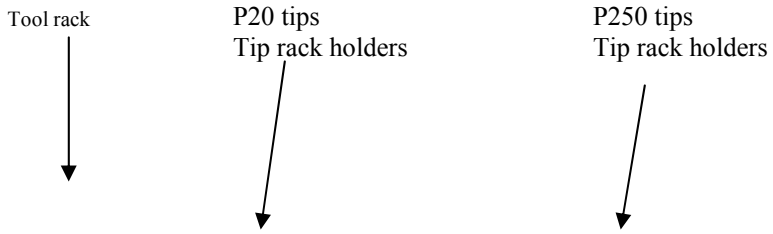
Date: 9/4/03
Author: B. Gold
Edited by: H. Yokota
Input: Cleaned-up PCR product plate and quantitation data
Output: Normalized plate, 1 picomole in 50 ul water
Next method: T4 polymerase reaction

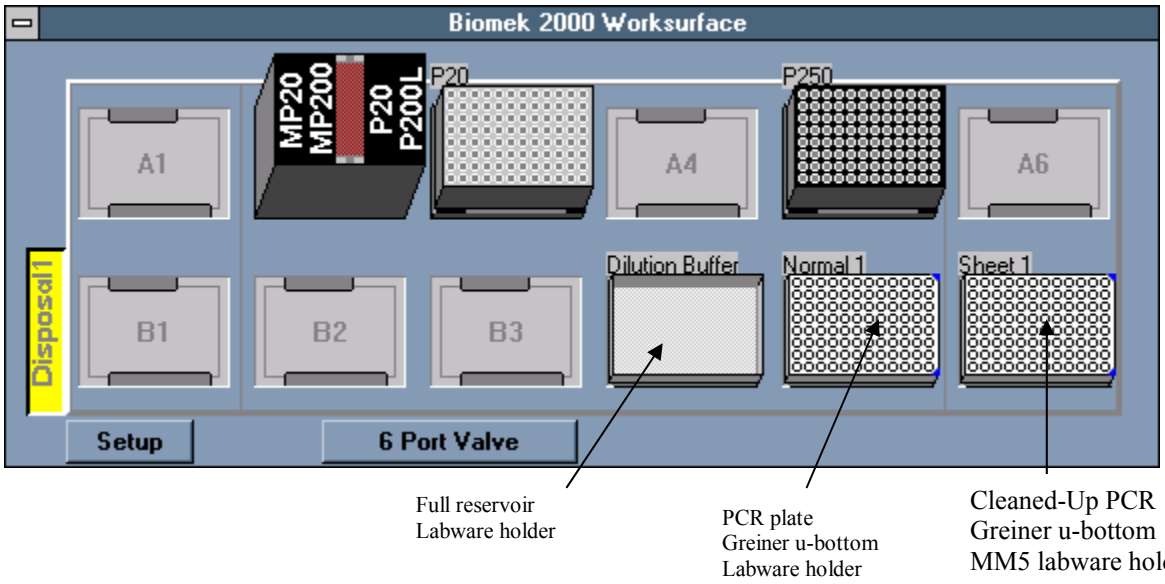
Materials/Reagents/Equipment	Vendor
Disposables	
PCR plate & mat	E&K (Cat#: 489096 & 402096)
Reagents	
Cleaned-up PCR product	Freezer
Water, sterile, for dilution reservoir	
Equipment	
Biomek 2000	Beckmann Coulter
Floppy disk w/ quantitation Excel table	

I. Importing Normalization Data

___1. Double click on Biomek Lab Book Manager icon on the desktop.
___2. Select the **Normalization** folder and click on **Set as Current Lab Book**. Click **Close.**
___3. Insert floppy disk with Normalization Excel file (see Picogreen Quantitation Protocol). Open My Computer on Desktop, double-click on Floppy (A:) drive.
___4. Open Normalization-date file, click File → Save as → Save in: Desktop → Normalization Test folder.

II. Setting up the Biomek worksurface





___1. Fill reservoir with autoclaved water.
___2. Place Cleaned-Up PCR Product plate on support Greiner u-bottom plate at B6.
___3. Place empty PCR plate on support Greiner u-bottom plate at B5.

III. Generating Normalization Method

___1. Click Start → Bioworks → Normalization Wizard → Next → Import plates.
___2. Look in: Desktop/Normalization Test folder.
___3. Select Normalization-date file and click Open: file becomes Sheet1 in Wizard.
___4. Click Next for the "Configure Options" window.
Change **Final Concentration** to **1.0**.
Change **Final Well Volume** to **50**.
___5. Click Next for "Configure Transfer" window, then Next for "View Plates"
___6. Click **Exclude Errors** to remove empty wells highlighted in red.
___7. Click Next for "Generate Method" window, OK to No Lids warning.
___8. Click **Generate Method**. The method is saved in Normalization Lab Book as Norm-date-hour.

IV. Editing and Running Method

___1. Click **View Method**.
___2. Click on Sheet1 at B6 to highlight plate in green. Press Delete key to remove.
___3. Click on **Devices** on Function Palette, scroll down and select **Norm MM5-B6**.
___4. Click on B6 to place Norm MM5-B6 (shaker labware holder).
___5. Click on **Plate**s on Function Palette, select **96-well u-bottom**, place on B6 holder.
___6. Click on tip boxes at A4 and A6 to highlight and press Delete.
___7. Click on the running man button to start the method. (Note: Save all the settings and click **Accept All** to confirm the configuration.)
___8. After the run, cover and label normalized plate (1 picomole in 50 ul), store in Freezer 318 until T4 polymerase digestion (see protocol).
___9. Cover and store Cleaned-Up PCR plate in Freezer 318.