

Biomek E-gel Procedure

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This method was developed to analyze the PCR screen of plasmid clones, with columns of 8 clones per gene. The 10 ul PCR screen rxn is diluted up to 25 ul in the PCR plate, because 20 ul are needed in each well of the E-gel. Empty wells must be loaded with 20 ul of water. The DNA standard is loaded manually. The Biomek loads the samples on the E-gel on the E-gel Motherbase, which is then plugged in for the electrophoresis. A U.V.digital picture is taken of the gel and saved on a disk for transfer to a computer for editing and analysis.

Input: PCR screen plate of plasmid miniprep

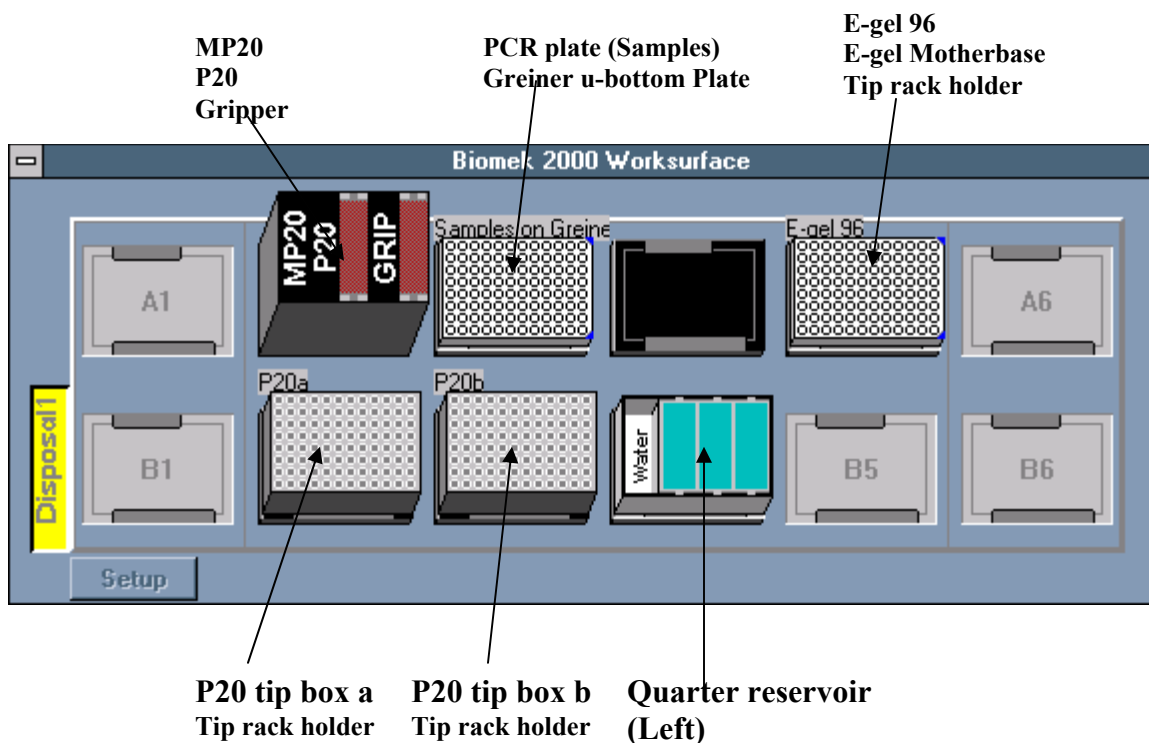
Output: Table of positives, i.e. clones with target insert

Reagents	Vendor
Low Range DNA Standard	Invitrogen (Cat#12373-031)
DNA Samples in 96-well PCR plate	Freezer318
Materials	
3 ¹ / ₂ in. Floppy disk	
E-gel	Invitrogen (Cat#67008-02)
1.5ml microfuge tube	
Equipment	
Biomek 2000	Beckmann Coulter
E-gel Motherbase	Invitrogen
Computer with Adobe Workshop & E-gel 96 Editor	Micron
Digital camera	UVP Bio Doc-It System
8-channel pipettor 250ul, non-sterile 250 ul tips	Matrix

Procedure

I. Preparation

___ 1. Set up the work surface of the Biomek as follows:



'Pbd-fs-1'(J:)/BSGC/BSGC Methods/PepecDB Methods Folder/Research/Protocol LIC

- __2. Fill the reservoir at B4 with water.
- __3. If DNA sample plate has empty wells, fill them with 20 ul water, with multichannel pipettor and Biomek reservoir.
- __4. Open E-gel package and remove comb from gel. Record E-Gel ID to link with DNA sample plate. Insert gel on base making sure the electrodes are in contact and the gel is firmly under the base electrodes.
- __5. Dilute 80 ul of DNA Standard with 80 ul water in 1.5 ml microfuge tuber tube. Using a pipettor, load 20 ul of the DNA Standard in each well of the last E-gel column. (column13, marked M)

II. Loading the gel with Biomek

- __1. Double click on **Biomek Lab Book Manager** icon on the computer Desktop.
- __2. Select the **E-GEL** 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 Load + Run E-gel 96**.
- __6. Click on the “running man” button on the Toolbar to start the method.
- __7. After running the method,, discard tips. Tip usage: both P20 racks.

III. Running the gel

- __1. Plug in motherbase (can be done on Biomek worksurface→ the Time LED display lights up, indicating running time; gel should be run 13-15 minutes, never more than 20. Time can be increased by pressing the Time button (holding it down will cause it to cycle back to 00).
- __2. Press Power button → LED changes from red to green → gel electrophoresis begins, the Time LED indicating the time remaining. An alarm sounds when the run is finished.
- __3. Unplug motherbase and remove E-gel.

IV. Saving the gel picture

- __1. Take E-Gel and a formatted Floppy disk to digital camera in Rm# 317. Put E-Gel in camera box, turn on Power and White Light to position gel in box and adjust focus until gel fills screen and ID number is legible.
- __2. Shut camera box door tightly by turning knob, turn off White Light and turn on UV switch (below door). The DNA bands should be visible below the wells. Adjust light intensity with + and – buttons. Press Capture.
- __3. Insert Floppy disk. Press Save. A filename for the .TIF image will appear at the bottom of the screen. Record filename.
- __4. Discard gel and take disk to computer in Rm#318.
- __5. Insert Floppy disk in A: drive. Click on **Start → Programs → Adobe → Photoshop → On Toolbar: File → Open → Desktop/ My computer/ Floppy (A:)/ filename**.
- __6. Click on **Image on Toolbar → Adjust → Brightness/Contrast** to increase sharpness of DNA bands.
- __7. Click on **File → Save as → Desktop / E-gel pictures / filename**. Scroll down Format field and select **JPEG**. Click Save and OK to JPEG parameters (.TIF files do not open in E-gel Editor).

V. Gel Analysis

- __1. Double-click on **E-gel 96 Editor** icon on Desktop. Click **File → Open → Desktop / E-gel pictures / filename**.
- __2. Follow Wizard prompts: Click on one of 3 red circles and, holding mouse key down, move it to center of closest bullseye on border of gel picture. Repeat with other 2 red circles. Click Next → click

Adjust to align red and green lanes → Click Next to display 96 sample lanes and 8 Marker lanes in 13x8 format.

__4. Click on lanes A1, B1, C1, D1, E1, F1, G1, H1, and HM to highlight the lanes in red frames, right-click on page for menu and click Copy.

__5. From the Desktop, open CompII Cloning/ Miniprep/ Egel template3. Save as Egel + plate ID. Click on first block of lanes, right-click for menu and click Paste.

__6. Repeat the copy and paste with columns 2 through 12.

__7. Enter the Target IDs and basepairs above the lanes (3 targets with basepairs can be copied and pasted together from a Target Summary Excel file).

__8. Analyze clone bands in relation to DNA Standard and enter positive clones as Xs below lanes.