

## Sequencing Protocol for the Beckman CEQ8000



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## I. Materials

1. Provided by the sequencing facility:
  - a. Sequencing kit
    1. Master mix
    2. Test primers and plasmid
    3. Sample loading solution
  - b. Glycogen
  - c. Mineral oil
  - d. Gel
  - e. Buffer
  - f. Buffer plates
  - g. Capillaries
2. Provided by the user:
  - a. Reaction tubes or plates
    1. Recommended products:
      - a. 0.2 thin-wall PCR tubes PGC #
      - b. Thin-walled microplates: Costar polypropylene plates  
Fisher #07200613, Product Code 6551
    - b. 100% molecular biology grade ethanol
    - c. 3 M Sodium Acetate (pH 5.2)
    - d. 100 mM Na-EDTA (pH 8.0)

## II. Costs

1. The sequencing costs are as follows:
  - a. Half reaction - \$5.00
    1. The standard reaction that generally gives an output of 500-700 bp
  - b. Full reaction - \$8.00
    1. This reaction may give outputs of 700-800 bp
  - c. Machine costs - \$2.50
    1. The cost of running empty wells in a column, for example, if you run 5 reactions on a plate, the machine costs apply to 3 wells (to fill the one column of 8).

### III. Sequencing Reaction

#### 1. Reaction Protocol

- a. Reactions should be put together in 0.2 mL thin wall tubes or 96 well microplates. All reagents should be kept on ice during the preparation.
- b. To the tubes, add the appropriate amount of template DNA (100 ng of plasmid DNA with 500-1500 bp inserts, for example) and water up to 5  $\mu\text{L}$ .
  1. Incubate the DNA at 95 °C for 1 minute.
    - a. This step is very important. It nicks the DNA so that the sequencing reaction can proceed. Missing this step will drastically reduce the quality of your results.
  2. Allow the reaction to cool before proceeding
- c. In the following order, add to the DNA:
  1. 1  $\mu\text{L}$  of 3.2  $\mu\text{M}$  primer
  2. 4  $\mu\text{L}$  of the DTCS Quick Start Master Mix
- d. Mix the components thoroughly and consolidate the reaction at the bottom of the tube by shaking or brief centrifugation

#### 2. Thermal cycling program

- a. 30 cycles of:
  1. 96 °C            20 seconds
  2. 50 °C            20 seconds
  3. 60 °C            4 minutes
- b. 4 °C hold

#### 3. Ethanol precipitation

- a. It is best to do the precipitation as soon as possible after the thermal cycling reaction has completed. Do not freeze the samples at this point, but allowing them to sit overnight at 4 °C is acceptable.
- b. Prepare fresh Stop Solution for each precipitation by mixing together 2  $\mu\text{L}$  of 3M sodium acetate (pH 5.2), 2  $\mu\text{L}$  of 100 mM  $\text{Na}_2\text{EDTA}$  (pH 8.0), and 1  $\mu\text{L}$  of 20 mg/mL glycogen for each sequencing reaction.

- c. Add 5  $\mu\text{L}$  of Stop Solution to each sequencing reaction and follow the appropriate precipitation protocol below. Vortex vigorously for 20-30 seconds.
- d. Using the appropriate protocol below, ethanol precipitate the DNA.

#### Tube precipitation

*If the reaction was done in 0.2 mL tubes, follow this protocol.*

- Add 60  $\mu\text{L}$  of ice cold 95% ethanol to each reaction. Mix thoroughly by vortexing about 10 seconds.
- If doing more precipitations than the centrifuge can hold, add the stop solution to all reactions, but wait until ready to do the entire precipitation to add the ethanol.
- Spin samples at 14,000 rpm (20,000 x g) at 4 C for 15 minutes.
- Carefully remove the supernatant with a pipette, being careful not to disturb the pellet, which may be slippery.
- Rinse the pellet once with 150  $\mu\text{L}$  of ice cold 70% ethanol. Spin at 14,000 rpm (20,000 x g) at 4 °C for 5 minutes.
- Carefully remove the supernatant with a pipette, and allow the pellet to dry either in the hood, or a speed-vac.

#### Plate precipitation

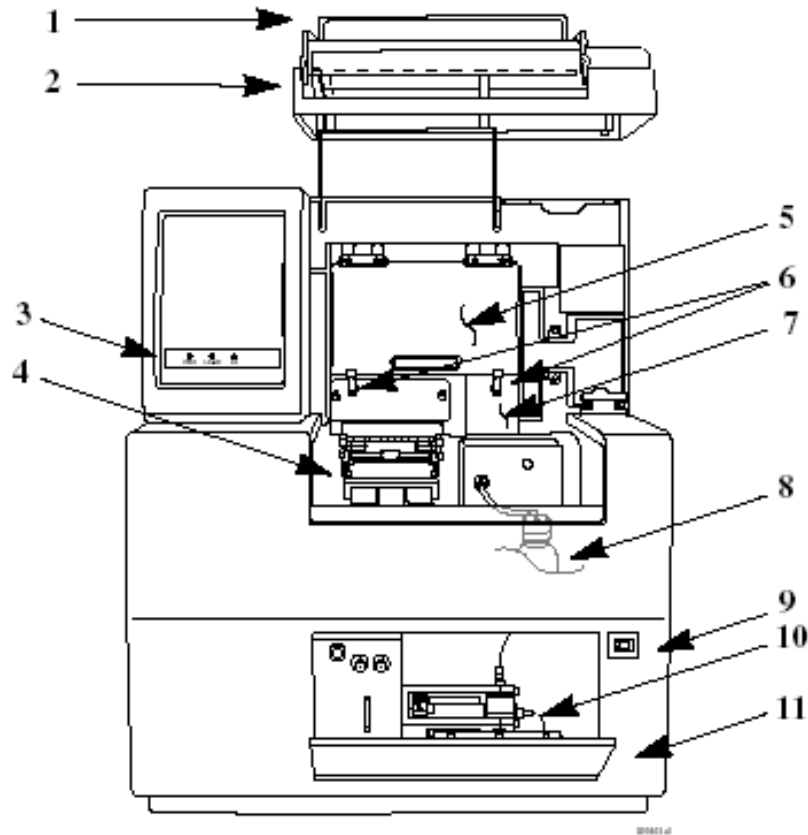
*If the reaction was done in microplates, follow this protocol.*

- Add 60  $\mu\text{L}$  of ice cold 95% ethanol to each reaction. Mix thoroughly by vortexing (about 1 minute).
- Spin samples at 5,000 rpm (4,200 x g) at 4 °C for 30 minutes. Carefully remove the supernatant with a pipette, being careful not to disturb the pellet, which may be slippery.
- Rinse the pellet once with 150  $\mu\text{L}$  of ice cold 70% ethanol. Spin at 5,000 rpm (4200 x g) at 4 °C for 30 minutes.
- Carefully remove the supernatant with a pipette, and allow the pellet to dry in the hood.

Resuspend the sample in 40  $\mu\text{L}$  of Sample Loading Solution (provided with the kit).

4. Preparing sample plates
  - a. Overlay 1 drop of mineral oil to each sample
  - b. To any unused wells in a column, add 40  $\mu\text{L}$  of water, and overlay a drop of mineral oil.
  - c. Prepare a buffer plate using the flat-bottomed culture plates. Fill the wells corresponding to samples in your sample plate  $^{2/3} - ^{3/4}$  full of buffer.
  - d. After the reaction, samples can be kept at  $-20\text{ }^{\circ}\text{C}$  and re-run on the machine if necessary.

#### IV. The CEQ Machine



**Figure 1: User Accessible Hardware Components**

- |   |                                     |    |                                     |
|---|-------------------------------------|----|-------------------------------------|
| 1 | Sample Access Cover (extended)      | 7  | Manifold Access Cover               |
| 2 | Capillary Access Cover (extended)   | 8  | Gel Waste Bottle                    |
| 3 | Status Indicators                   | 9  | Power Switch                        |
| 4 | Plate Holders & Sample Transport    | 10 | Gel Pump                            |
| 5 | Capillary Temperature Control Cover | 11 | Gel Pump/Gel Cartridge Access Cover |
| 6 | Rubber Latches                      |    |                                     |

Figure 1: Accessible hardware components of the CEQ8000 (from the Customer Training Guide)

## II. Opening the CEQ8000 Software

1. Turn on PC and wait for windows to start
2. If not already on, press power switch to ON (Fig.1: #9)
3. Click the CEQ8000 icon on the desktop to open the window shown in the picture
4. There are 7 different modules available on this screen, most of which will be discussed later.
  - a. Sample Setup – used to set up the sample plates
  - b. Run – used to interact with the sequencing machines, and actually running your sequences
  - c. Sequence Analysis – used to look at your results
  - d. CEQuence Investigator
  - e. Fragment Analysis
  - f. Databases – manages where data is stored
  - g. Exit – exits the program

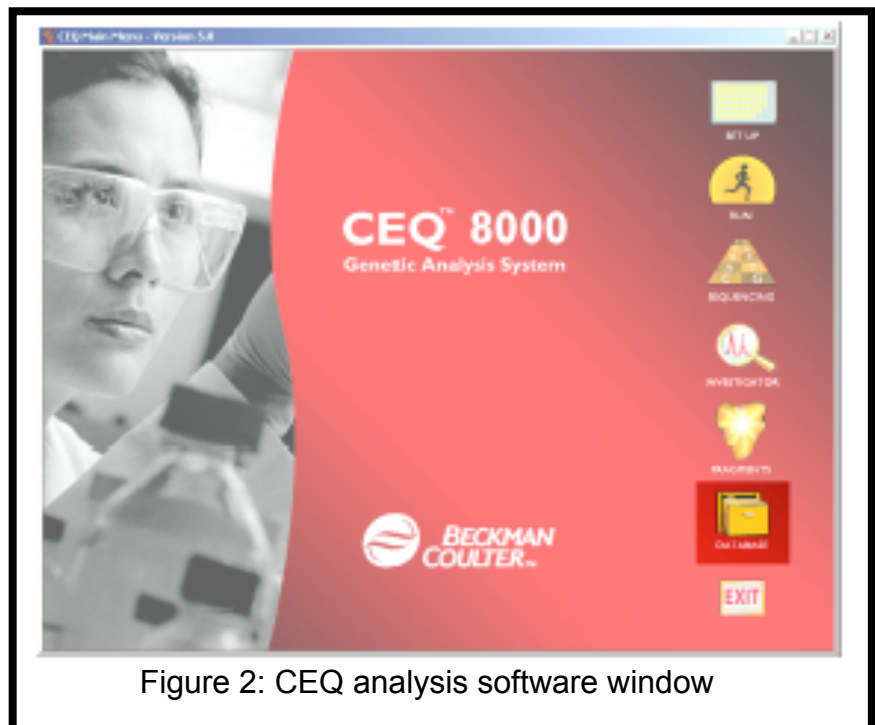


Figure 2: CEQ analysis software window



## II. Databases

1. From the CEQ analysis software menu, select the **DATABASE** module icon (Highlighted in Figure 2)
2. If you **do not** already have a database:
  - a. In the data manager module window (Fig. 3), select **File / New Database**
  - b. In the **New Database** dialog box:
    1. Enter the name for this new database
    2. Select **Set as Working Database** and click **OK**
  - c. If you wish to further subdivide data storage (otherwise, all the data is stored in the default project folder), create a new project folder:
    1. Highlight the desired database where the project will be
    2. Select **File / New**
    3. Highlight the new project and select **File / Rename**, enter in the desired name
3. If you **do** have a database:
  - a. Select **File / Set** or **right click** on the database and click on **Set As Working Database**
4. For the **Set As Working Database** option to be available, all other CEQ8000 programs, such as the run window, must be closed.

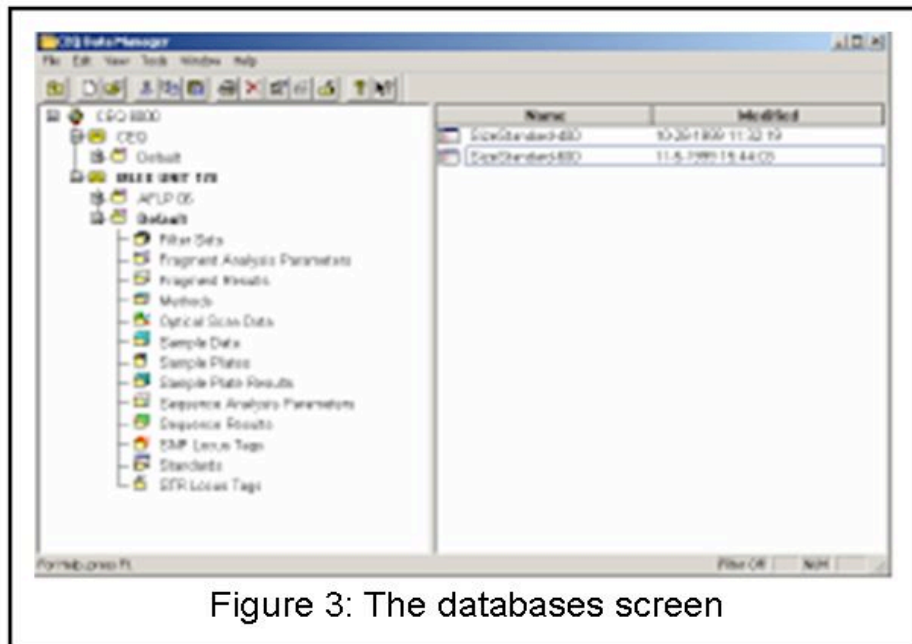
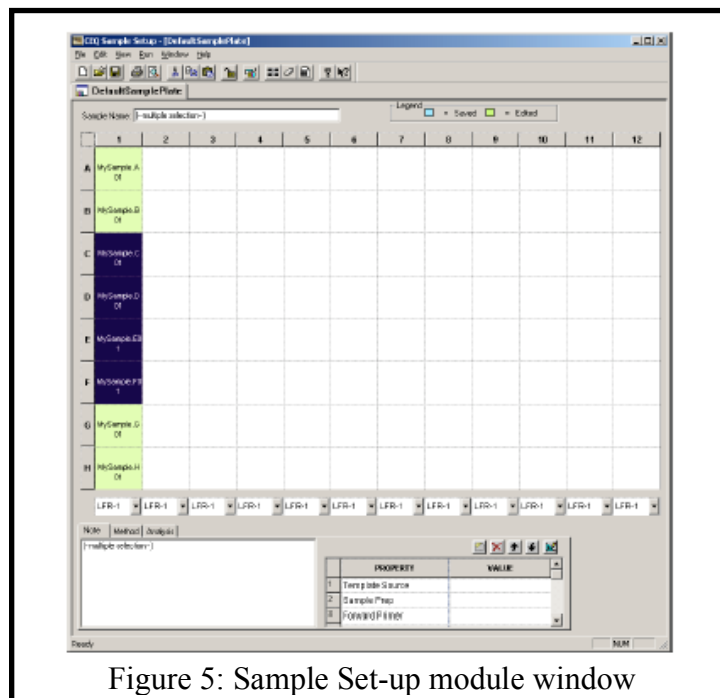


Figure 3: The databases screen

### III. Setting up the sample plate

1. From the CEQ Analysis Software window or the Shortcut Bar (Fig. 4), click the **SETUP** icon (circled in Figure 4).
2. In the plate set-up screen that appears, name the cells corresponding to the samples in your actual sample plate (Fig.6)
3. When making changes to the entire plate, be sure to highlight all of the cells.
4. Select a method from the drop-down menu at the bottom of the sample set.
5. Select the appropriate analysis parameters (as well as report and export parameters if needed).
  - a. Select both raw and analyzed data boxes to save the chromatograms as .scf (standard chromatogram files).
  - b. If you forget to export your data at this time, you can do it later.
6. Select **File / Save as**, you must save your work before you can continue! Saved cells will change color.
7. If needed, select the project you created in the database module from the **Project Name** drop-down menu and click **OK**.

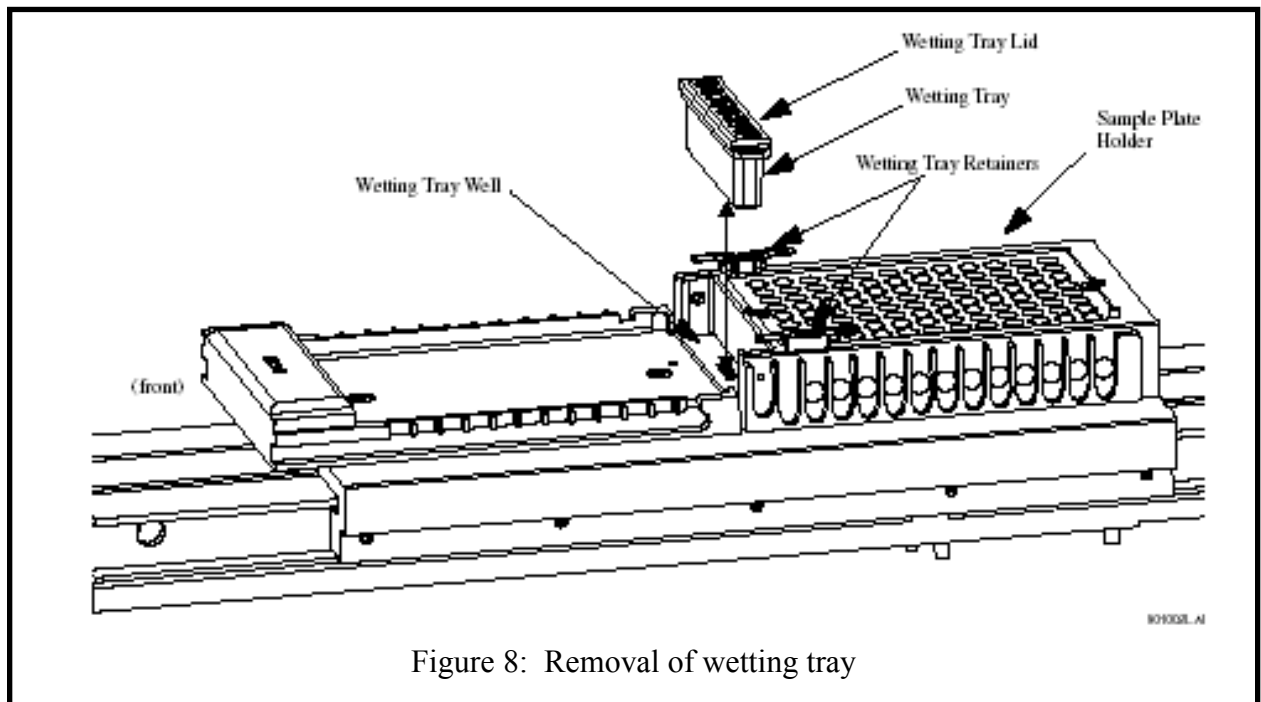
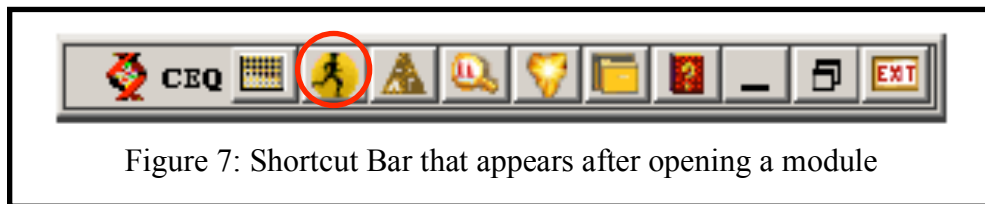


#### IV. Preparing the machine to sequence

##### 1. Wetting tray

- a. From the CEQ Analysis Software window or the Shortcut Bar (Fig. 7), click on the **Run** icon
- b. Click **Replenish / Replace Wetting Tray**
- c. Open the sample cover (Fig.1: #1) when prompted by the software.

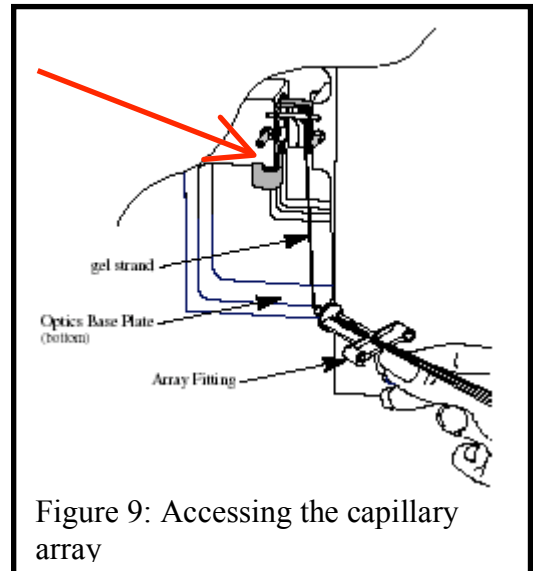
Remove the wetting tray (Fig. 8), empty any water in the tray and fill with distilled water up to the bottom of the letters, NOT TO THE FILL LINE.



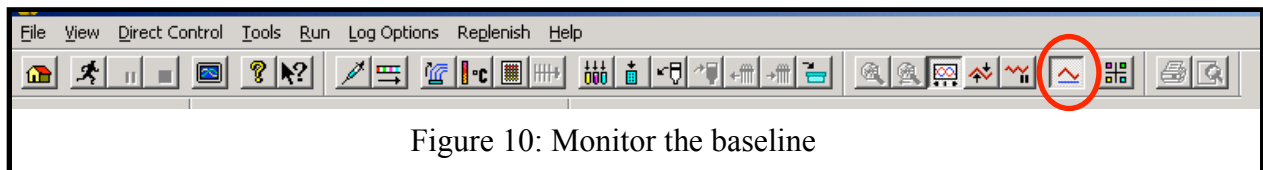
## 2. Capillary array

### a. Installation

1. Click on **Replenish / Release Manifold Plug** and the **Remove Capillary Array** dialog box will appear
2. Remove the manifold plug by opening the sample access cover (Fig. 1: #1), then open the capillary access cover (Fig. 1: #2) by placing hands where the plastic clear cover is latched and pull up
3. Unlatch the two rubber latches (Fig. 1: #6) holding the capillary temperature control cover (Fig. 1: #5) and lift it up
4. Loosen the first screw on the manifold access cover (Fig. 1: #7) and remove the cover
5. Loosen the two screws to the plenum (under the capillary temperature control cover) and remove that cover as well
6. On the right of the machine will be a red eject lever (where the manifold access cover was removed; Fig. 9), pull it up to release the manifold plug
7. When you pull out the array plug, hold it in place for 10 seconds (until the acrylamide gel strand solidifies) and then remove the manifold plug
8. Make sure to wipe off all gel that may be on or around the manifold plug area with a Kimwipe
9. Open the new capillary array and remove all the coverings.
10. Hold the capillary and the array by holding the protruding tabs.
11. Place the capillaries in the wetting tray while inserting the array into the array fitting.
12. Then insert the capillaries with tab pointing out.



13. Put the array fitting cover back on and screw it in.
  14. Take your left hand and put the capillary cover back on, while hold the capillaries in the notch where the cover closes with your right hand.
  15. Screw the capillary cover back on and then finally close the capillary access cover and secure the two rubber latches.
  16. Once the capillary is install hit **OK** on the dialog box
  17. After installing a new capillary array, be sure to purge the manifold (see page 16).
- h. Life span
12. Beckman recommends that the capillary array should be changed after 60 days, or 100 runs, whichever happens first.
- i. Monitoring the baseline
12. To ensure accurate analysis of the sequencing runs, monitor the baseline before each run.
  13. Click **Run, monitor baseline**. When a dialog appears, check the box next to **enable monitor baseline**. Or click on the button circled (Figure 10).



14. To view the baseline, click on the side tabs, A-H (Figure 11) one at a time. This will show you the baseline fluorescence readings. Wait around 30 seconds to let the readings even out.
  - a. There may be a spike in the baseline at the beginning of a run. If so, drag a square with the mouse to zoom in on the part that you're interested in.

15. If any of the baselines are greater than 6000 counts, try the following:
- a. An optical alignment
    - i. At the **direct control** screen, click on the optical alignment.
  - b. Clean off the array
    - i. Access the capillary array as above.
    - ii. Using a slightly damp Kimwipe, very gently clean off the capillary window.
  - c. Refill the capillaries
16. Recheck the baselines as above

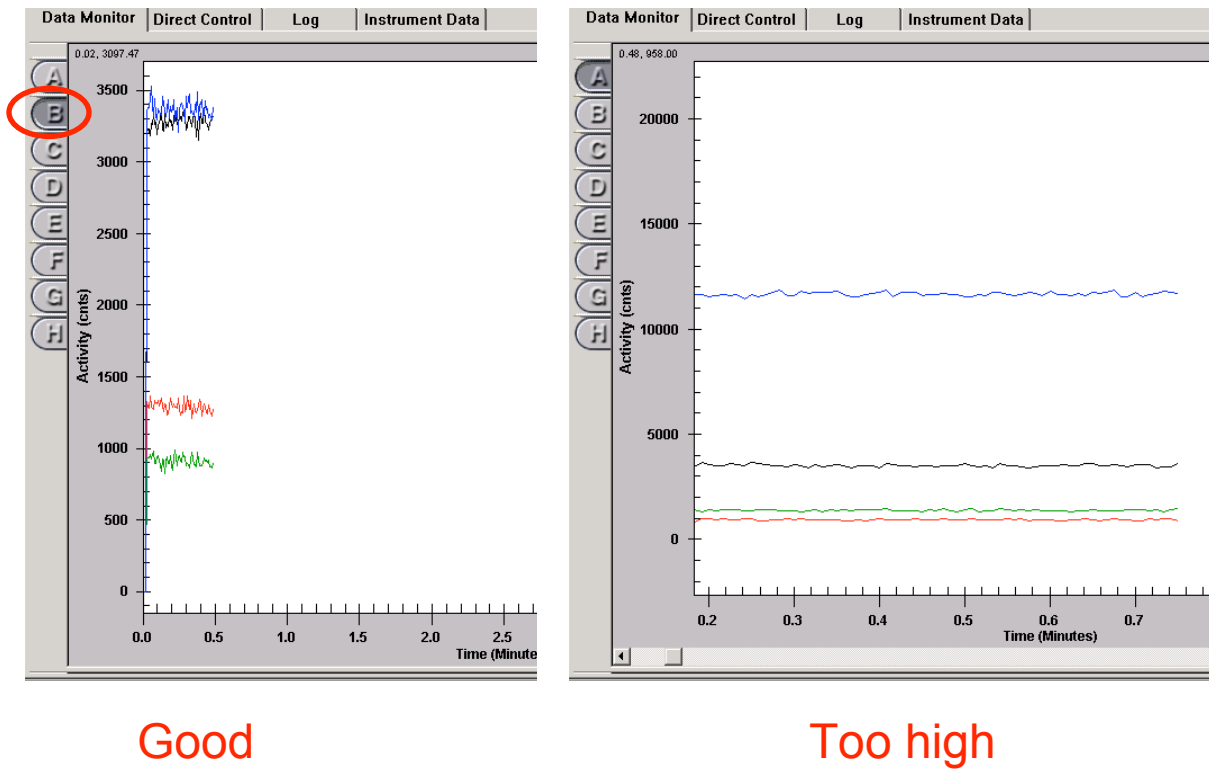


Figure 11

## Gel Cartridge

### a. Installation

1. Select **Replenish / Release Gel Cartridge**
2. When the system is ready to release the gel cartridge, the **Release Gel Cartridge** dialog box will be displayed. Wait until the lead screw is completely disengaged.
3. Open the gel pump access cover (next to the **On/off** switch) by gently pushing on it (it is spring-loaded)
4. Pull on the cartridge locking lever (it will pull outward to a 90 degree angle).
5. Grasp the wings of the gel plug and pull it outwards, wipe off any gel that may remain on the plug or in the gel cartridge.
6. Take cap off gel cartridge (wipe off any gel), place it on plug and place plug off to the side.
7. Take gel cartridge and insert it where the plug was.
8. Push the cartridge locking lever towards the back of the instrument into is locked position
9. Close the gel cartridge access cover.
10. In the **Remove Gel Cartridge** dialog box click on **install cartridge**, enter in the appropriate information and click **Done**.

4. Running the samples
  - a. Then go to **Direct Control**, click **purge manifold**.
  - b. After manifold is purged click under **Direct Control**, **gel capillary fill**
  - c. After it is filled, also under the same window, hit **optical alignment**
  - d. Then go to **Run, Monitor Baseline**, when its dialog box appears, check **enable monitor baseline**
    1. If any baselines are above 6000, try another optical alignment, or clean off the array, or purge the manifold
  - e. Then under the **Direct Control** window, click **Load sample plates**.
  - f. Load in your sample and buffer plates in the appropriate place (Fig. 8)
  - g. Then go into **Run, start sample plate** to begin your run. Make sure that the correct cells are highlighted
    1. The columns each take between 90 and 110 minutes to run.
5. Disassembly of the CEQ8000 - after your sequences have finished
  - a. Remove the sample and buffer plates from the machine
  - b. Rinse the wetting tray with distilled water and refill it
  - c. Remove the gel cartridge, using a Kimwipe to remove the excess gel from the end. Replace the cartridge with the plug.



### Sequencing checklist:

- Prepare sample plates with sequencing reactions.
- Fill any wells of incomplete columns in the sample plate.
- Cover all wells with mineral oil.
- Fill corresponding wells in the buffer plate with buffer.
- Create a sample plate.
  - ◇ Export the data.
  - ◇ Apply analysis and sequencing methods to all wells.
- Load the gel cartridge into the machine.
- Purge manifold.
- Check the baseline readings of the capillary array.
- Refill the wetting tray.
- Fill out the sequencing log on the computer.
- Start sample plate.

### Disassembly:

- Remove the sample and buffer plates.
- Rinse the wetting tray with distilled water and refill it.
- Replace the gel cartridge with the plug.
- Record the usage of the gel on the package and put it in the fridge.