# Automated Sequencing: Capillary Electrophoresis

**Please read first!!!**

Once your samples are dried down, your task is done!!! The following pages contain information on how your samples are prepared and electrophoresed for you. Once your samples are electrophoresed, the results will be e-mailed to you.

**Introduction:**

**Automated Sequencing:** The most current way of distinguishing among DNA fragments is by tagging the 4 types of ddNTPs with 4 different color dyes. For example, thymine = red, adenine = green, cytosine = blue, guanine = yellow. This was performed in *Investigation 13*. The automated sequencing method requires samples to be electrophoresed in a capillary tube containing a liquid polymer that contains acrylamide and urea. The sample is placed in a tray, and each base type can be distinguished from each other by the difference in its color that it fluoresces. As the DNA fragments reach the laser beam, the dyes become fluoresced as the laser beam scans the polymer in the capillary. The fluorescent light images are then converted to computerized images. The results come in both text and graphical form, showing the intensities of the bands as different colored peaks.



This method is safer to use than those using radioactivity, it can run up to 36 samples in a tray, electrophoresis one sample at a time, and only takes 45 minutes to generate a short sequence (around 300 bases) or less than 3 hours for a long read (about 800 bases). Since, this method is relatively quick, it is soon to replace the long vertical gel sequencers, where 36 samples are run at one time, but takes 18 hours to generate a sequence (since all samples are electrophoresed at the same time.) Data is saved as a computer file. The class will then view the sequence images using the Mac-based Sequence Navigator program in *Investigation 15*.

**Materials:**

micropipettors & tips microfuge thermocycler (95°C)

waste beaker vortexer gloves

dsH2O kimwipes septa

TSR microtube rack & marker timer

0.5 ml capless tubes ice bucket

**Procedure:**

## Prepare Sequencing Samples:

1) Fill your ice bucket and gather your DNA samples from the freezer.

2) **Put on gloves**. Set up tubes. Label 0.5 ml capless tubes with numbers assigned to you by the instructor. Place the numbers at the top of the side of the tube to be written horizontally as you hold each tube vertically. Keep a key in your lab notebook as to what sample each number represents.

 Example:

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3) Add **25 μl** of **TSR** (template suppression reagent) to each **DNA sample**. Pipette up and down to assure the DNA dissolves into the reagent. **Change tips each time!!!** Vortex two at a time (one in each hand) and pulse down briefly.

4) Transfer each sample to the appropriately labeled capless tube, changing tips each time. Place a rubber septum on top of each tube. Make sure they are on all the way and not twisted.

5) Place samples in the **95°C** thermocycler. Using your timer, heat for **2 min**, then place immediately on ice.

6) After at least **5 min**, place your samples in the gray loading tray in numerical order, according to the instructor’s guidelines.

The gray tray contains rows of holes that are offset every other row. The first row is labeled “A” and with odd numbers, the second row is labeled “B” and with even numbers, etc…The instructor will place sample #1 in A1, sample #2 A3, sample #3 in A5, sample #4 in A7, sample #5 in A9, sample #6 in A11, sample #7 in the next row B2, sample #8 in B4, and so on. The tray button in the sequencer is pushed to gain access to the sample area. The gray sample tray is popped into place so that the A1 hole is at the far right corner. (Tray is turned 90° clockwise at this point.)

**Prepare and Load 1X Buffer and dH2O**:

1) Using a green top pipette, add 1500 μl of 10X buffer to a 125 ml bottle.

Using a Pipette-Aid, add 13.5 ml of dsH2O to the bottle and mix by inversion.

2) Add buffer up to the red line on the shot glass (for positive electrode).

3) Add buffer to the black line of the glass tube that is located at the far left of the sequencing tray. Place the white cap with septum on top of the tube, and place in the far left hole of the tray.

**Prime and Load Syringe with Polymer**:

1) Take polymer out of 4°C and bring to room temp. Wash the 1ml glass syringe with warm water and rinse with dH2O. Use the plunger to get rid of excess water. Clean the O ring with ferrel and place back on the tip. Prime the syringe with a small amount of drawing up a small amount. Take syringe out of the polymer bottle and pull the plunger most of the way out to coat the sides with the polymer. Then dispense the polymer in the sink. This will get rid of excess water. Wipe the end of the syringe with a kimwipe.

2) Fill syringe with polymer (~0.8ml). Using the water bottle, squeeze some dH2O on the tip of the syringe and wipe with a kimwipe.

3) Invert syringe and tap it to allow any air bubbles to rise up to the tip. Then purge the plunger to get rid of excess air.

4) Squirt dH2O on the outside of the syringe and wipe with a kimwipe to remove excess polymer.

5) Use manual control to put the machine’s metal **syringe up** to gain access to the syringe area.

6) Screw in the glass syringe and make sure the upper left ferrel is closed.

7) Use manual control to **close buffer valve**. Open the waste valve at the bottom of the block by unscrewing the ferrel three turns. Then squeeze the plunger down to fill a drop in the waste area. This is to get rid of the air bubble. Then close the bottom ferrel.

8) Use manual control to **open buffer valve**. Push down on plunger to fill block with polymer. Keep pushing until it is full and all air bubbles are out.

9) Use manual control to **close buffer valve** and **lower syringe** plunger until it is hugging the top of the glass syringe’s black plunger. Use 2-5 increments at the end for fine adjustment.

**Fill Out Sample Sheet:**

The instructor has double-clicked on ABI 310 collection icon to launch the program. Under file: **NEW** was chosen, and **sequence sample sheet 48 tube** icon launched.

1) Type in the name of your sample (including the primer name). For example, W01F5-Osp2’.

2) In the dye set column, Choose **DT310POP4(BD)v1.mob** if doing a short read, or **DTPOP6(any primer set)** if doing a long read.

 DT=dye terminator,

310=model of machine,

POP4=4%performance optimization polymer

BD=Big Dye cycle-sequencing reagents

3) In the matrix column, choose **bd matrix**.

bd=big dye cycle-sequence reagents

4) Save sample sheet. The file is automatically put into the genetic analyzer **sample sheet folder** on the hard drive.

**Fill Out Injection List:**

Once all of the students have filled in the sample sheet, the instructor will choose **NEW** under file, and double-click on the **sequence injection list** icon. In instructor then chooses the **sample sheet**. It will be today’s date sample sheet in the pop-up menu. The first column automatically fills in. The module **SeqPOP6(1ml)E** for a long read, or **P4stdseq(1ml)E** for a short read is then chosen for the first sample in the next column.

 P4=POP4

stdseq=standard sequencing

1ml=size of the machine’s syringe that holds the polymer

E=type of matrix built for this machine

The rest of the samples’ modules are then chosen by highlighting the module of the first sample, holding the shift key down and clicking on the last sample’s second column to highlight the entire column, then chosing under edit: **fill down**, to fill in the rest of the boxes in that column. All of the other columns are automatically filled in. When ready, the instructor will then click on **RUN**. It will take the machine several minutes to reach 50°C, then the polymer in the capillary is replaced, and the first sample is electrophoresed.