Colby J. Res. Meth. 2008. 11:10-13

Electrophoresis of RNA (and the Joy of Pipeting)

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week #4 (04 March)

I. First - the Joy of Pipeting

For many techniques that are now common in modern molecular biology, it is essential to have excellent pipeting skills. Often, very small volumes must be transferred with great accuracy and repeatability. This will be true for the dilution of RNA samples that you will carry out today, and it is especially true for the quantitative RT-PCR that you will carry out next week.

For this reason, we will spend the first part of today's lab period honing our skills in pipeting. It is assumed that you already have some experience in the use of adjustable pipets. Today's focus will be on accuracy and repeatability when pipeting small volumes.

This part of the lab will be done individually, not with a lab partner

EXPERIMENTAL PROCEDURE

A. Checking your pipets

1. You will need to have a beaker of water, a P10, a P200, a P1000, and a scale.
2. Place a weighing dish on the scale and set the scale's readout to zero.
3. Set the P1000 to deliver 1000 μL, and carefully pipet "1000 μL" of water onto the weigh dish. *Record the weight of the water.*
4. Using the same system, test the P1000 at 500 μL and at 200 μL. *Record the weights of the water.*
5. Test your P200 at 200 μL and at 25 μL. *Record the weights of the water.*
6. Test your P10 at 10 μL. *Record the weight of the water.*
Did your pipets deliver the volume of water that they were supposed to? If any of your pipets has a significant problem, it should either be cleaned or sent back to the factory for recalibration.

B. Repeatability in delivering a small volume

1. Obtain 6 semimicro spectrophotometer cuvets. Using your P1000, place 1000 μL of water into each cuvet. Label one of these cuvets as a blank.
2. Carefully pipet 3 μL of Solution B into each of the other five cuvets. Mix the solution in each cuvet by inversion, covering the top with a piece of parafilm.
3. Following the instructions in Appendix B, measure the absorbance of each solution. *Record these values in your notebook.*
Did all five of your solutions have the same absorbance? If not, how different were they? Would this amount of variability in pipeting have a negative effect on a real experiment?

C. Delivering different volumes

1. Obtain 6 new semimicro spectrophotometer cuvets. Label these cuvets as #1 through #6. Place 2000 μL of water into cuvet #1 (which will be the blank).
2. Into cuvets #2 through #6, deliver 2 μL, 5 μL, 10μL, 15 μL, and 20 μL, respectively, of Solution B. Add water to each cuvet to give a total volume of 2000 μL. Mix the solution in each cuvet by inversion, covering the top with a piece of parafilm.

3. Following the instructions in Appendix B, measure the absorbance of each solution. *Record these values in your notebook.*
Was the absorbance of each solution proportional to the amount of solution B present? If not, how serious were the discrepancies? Would this amount of variability in pipeting have a negative effect on a real experiment?

APPENDIX B

MEASURING THE ABSORBANCE OF BLUE SOLUTIONS USING THE DU530 SPECTROPHOTOMETER

1. Turn on the spectrophotometer and allow it to go through its warm-up procedures.

2. From the Main Menu, Select Fixed λ.

(3). If the wavelength is not already set to 595 nm then do this: Select Options, then GOTO λ, λ_1. Type in the λ that you want (595) and push the Enter key.

4. The screen should now state that the λ is 595 nm and near the bottom it should say "Blank 1; Read 2-6".

5. Place your cuvets into the multicell module of the spectrophotometer. Your blank should be in positon 1 and your samples in positions 2 through 6. Make sure the cuvets are oriented so that the 1 cm light path goes from left to right when the cuvet is in the position to be read.

6. Push START to read the absorbance of your samples. After all the readings have been made, you can scroll up and down (using the arrow keys) to see the absorbance for each cell. Write down the values.

Solution B

50 µL 1 M Tris pH 8
4950 µL dihydrogen oxide
10 mg bromophenol blue
5000 µL

Once you are satisfied with your skills in pipeting small volumes, get together with your lab partner for the *RBCS* gene expression experiment.

II. Second - the Electrophoresis of RNA

To confirm that you have isolated RNA that is pure and not degraded, you will analyze your RNA samples on an agarose gel.

Rather than prepare your own agarose gel (which is fun but takes up time), you will use a type of premade gel (an RNA FlashGel) that is designed for separating RNA. The loading buffer and the RNA FlashGel contain denaturants (eg. formaldehyde) to make sure that the RNA remains single stranded throughout the run. This ensures that the mobility of RNA molecules will be proportional to their size and not influenced by things like secondary structure.

EXPERIMENTAL PROCEDURE

Preparation of diluted RNA samples

For each of your RNA samples (purified last week), prepare 10 μL of a 200 ng/μL dilution (for this week's gel). Also prepare 100 μL of a 20 ng/μL dilution of each sample and store this in the freezer for next week. RNase-free water should be used for making these dilutions.

Preparation of RNA samples for the gel

To prepare each RNA sample for running on the gel, mix the following components:

500 ng RNA	2.5 μL of the 200 ng/μL sample
2x FS buffer	2.5 μL
	5.0 μL total

Mix the samples gently and incubate them at 65° for 5 minutes. Place the samples on ice. Spin the samples briefly to make sure all the liquid is at the bottom of the tubes.

Running the gel

Remove the RNA FlashGel from the package and remove the white tapes that cover up the wells and buffer chambers. Each gel contains 13 wells, which will be enough for two groups (6 samples + 1 RNA standard + 6 samples). Insert the gel into the FlashGel dock. Fill each of the wells with water (about 20 μL). Load your samples (5 μL of each) into the wells.

Run the gel at 225 V for 5 minutes. Do not touch the FlashGel dock or gel while the voltage is turned on.

Photographing the gel

After the gel is finished running, take a photograph of your gel with UV illumination (using the camera in Arey 204). Print out a good photograph of your gel that also includes the lane of RNA standards.

Also save a jpeg file of your gel photo on the desktop of the computer (in the BC378 folder). Open the Academic Server (smb://fileserver1/academic) and copy your file into the BC378 folder on the Academic Server. You will now be able to access this file later from any computer on the Colby network.

Include the photo of your gel (with all lanes clearly labeled) in your lab notebook.

Look closely at the photograph of your RNA gel. *What species of RNA molecules can you see in the photo? Does your RNA appear to be degraded? Is there any DNA mixed in with your RNA? Is there an equal amount of RNA in each of your samples?*

Note: in the lane where you ran the RNA standards, you should see six bands. These correspond to 9.0 kb, 5.0, 3.0, 1.5, 1.0. and 0.5. kb.

2x FS Buffer

200 µL 5x FGR buffer
1200 µL 37% formaldehyde
1000 µL formamide
1 mg bromophenol blue
1 mg xylene cyanol
3000 µL