Electrophoresis of TMP-PCR Products

Today you will run your PCR products on a 2% agarose gel. Since you have 16 samples (plus DNA ladder), you will run a gel with two 15-well combs. (Be aware that it will take more microwaving to get this much agarose into solution.) From the results, you should get a yes or no answer for each primer, i.e., did the primer amplify part of the TMP gene from your phage DNA? You can also calculate the size of the amplified DNA fragment.

The steps for today are:

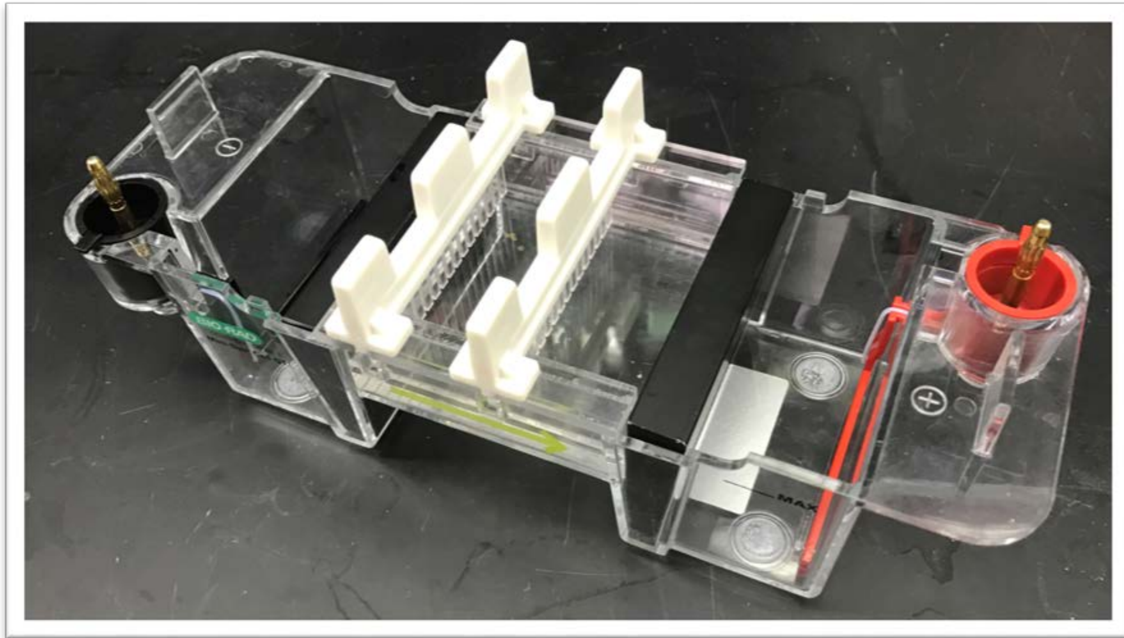
1. Prepare a 2% agarose gel– each person will have their own gel
2. Add loading dye to your PCR reactions
3. Load the samples and a DNA ladder on the gel
4. Run the gel and analyze the results.

Materials per ***person***

Item	**Quantity**
Bio-Rad gel box with two 15-well combs	1
10x TAE (in lab drawer)	3 ml
agarose	0.6 g
GelRed (in lab drawer)	3 µl
6x loading dye (in lab drawer)	64 µl
floating rack	1
lid locks	6
Promega PCR molecular weight ruler	30 µl
0.25x TAE buffer	300 ml
plastic box	1
hot hands grippers (shared)	1
250 ml flask	1

Procedure 1: Preparing the gel

1. Prepare gel box by placing the gel tray and buffer dams in position. Place 2 combs in slots.



2. You are preparing a 30 ml 2% agarose gel in 1x TAE buffer.
    a. Place an Erlenmeyer flask on the balance and tare to zero. Weigh 0.6 g of agarose directly into the flask.
    b. Add 3 ml of 10x TAE and 27 ml of distilled water to the flask.
    c. Tare the balance to zero and weigh the flask and its contents.

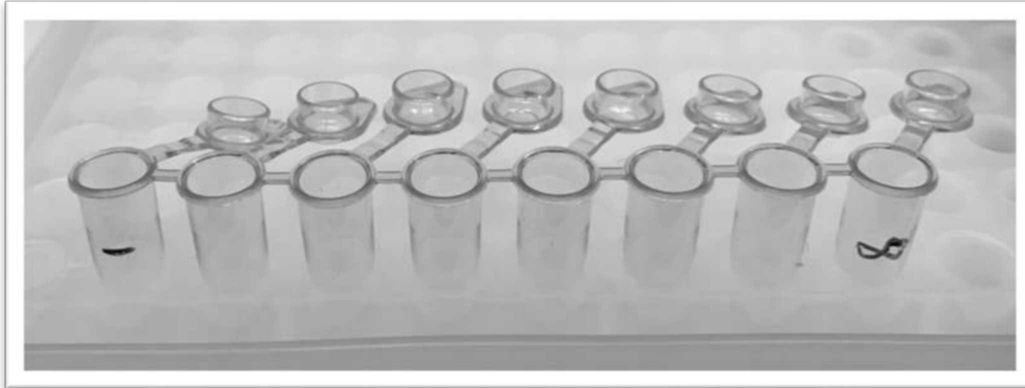
    Record the weight: ______________

3. Heat the solution in the microwave on high for 45 s. Carefully swirl the solution to mix, then microwave for an additional 20 s. Use the thermal grippers and be very careful, as the solution may be superheated. Check that all of the agarose is in solution. If not, microwave for an additional 10–20 s.
4. Tare the balance to zero and weigh the flask and contents. Add distilled water to bring the weight of the solution and flask back to the original weight and swirl to mix.
5. Add 3 µl of GelRed to the agarose solution. Swirl flask to mix, and pour solution into the gel box. Allow gel to solidify.

    While the gel is solidifying, go on to procedure 2 and prepare your samples.

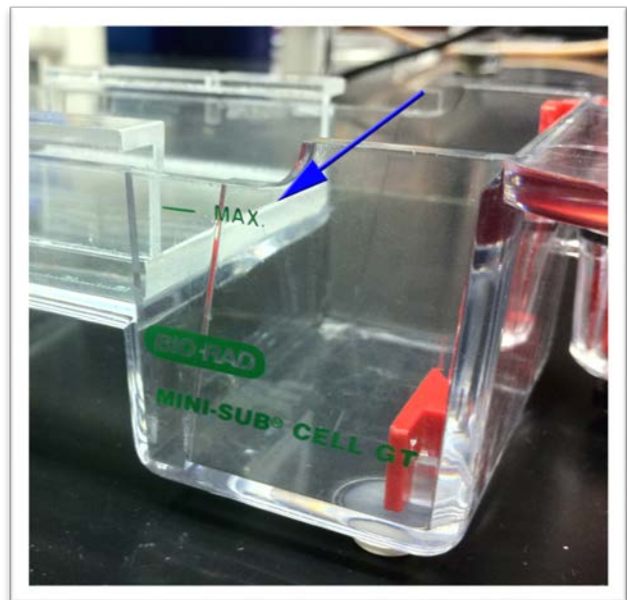
Procedure 2: Preparing your samples and running the gel

1. Get your 2 PCR strips from the front of the room. Remember to orient them correctly, with the lids slanting to the right. The blue strip contains reactions 1–8 and the pink strip has samples 9–14 plus controls.



2. To each tube, add 4 µl of 6x loading dye. Mix, then spin strips briefly to bring contents to the bottom of the tubes.
3. Get a tube of Promega PCR Molecular Weight Ruler (it is ready to load) and a beaker of 0.25x TAE buffer (~300 ml).
4. When the gel has solidified (it will look opaque), carefully remove the buffer dams and combs. Note: be careful not to misplace the combs—they are very expensive!
5. Fill the gel box to the "Max" line with 0.25x TAE. The buffer should cover the gel completely.
6. Load your samples in the following order using a P20 pipettor.

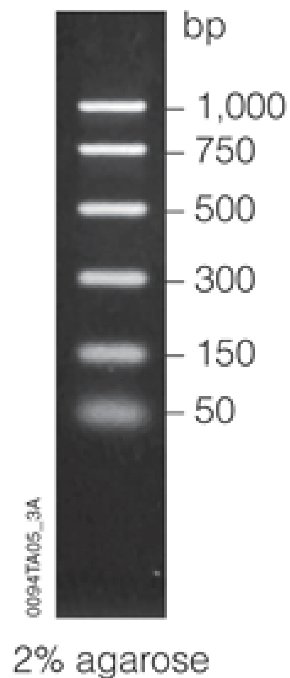
   Use the first row of wells for samples **1–8**, and the second set of wells for samples **9–14 and controls**. Both sets of wells will also have a molecular weight standard.



Lane	Sample	Load volume		Lane	Sample	Load volume
1	PCR MW Ruler	10 µl		1	PCR MW Ruler	10 µl
2	**PCR Tube 1: A1**	5 µl		2	**PCR Tube 9: E**	5 µl
3	**PCR Tube 2: A2**	5 µl		3	**PCR Tube 10: F1**	5 µl
4	**PCR Tube 3: A3**	5 µl		4	**PCR Tube 11: F2**	5 µl
5	**PCR Tube 4: A4**	5 µl		5	**PCR Tube 12: G**	5 µl
6	**PCR Tube 5: A5 mix**	5 µl		6	**PCR Tube 13: K**	5 µl
7	**PCR Tube 6: A6**	5 µl		7	**PCR Tube 14: N**	5 µl
8	**PCR Tube 7: B1**	5 µl		8	**PCR Tube 15: positive**	5 µl
9	**PCR Tube 8: B3**	5 µl		9	**PCR Tube 16: negative**	5 µl
10				10		
11				11		
12				12		
13				13		
14				14		
15				15		

7. Run gel at 150 volts for 20–30 min.
8. Take your PCR tube strips to your TA for storage at –20˚C (in case you have to re-run the gel).
9. Place the gel and casting tray in a plastic box and take it to the GelDoc for imaging.
10. After you have imaged the gel with the GelDoc, take a cell phone image of the gel on the screen for a quick analysis. (See following page for analysis.)
11. Dispose of the gel in the large blue waste container.
12. Clean-up
    a. Rinse gel box, comb, and casting blocks with distilled water and place on bench paper as instructed by your GTA.
    b. Place dirty beaker, flask, and graduated cylinder in the dish pans.
    c. Keep any remaining 10x TAE, loading dye and GelRed in your drawer.

Promega PCR Molecular Weight Ruler



Analysis of Results

Effects of temperature on phage replication

Your plates were removed from the incubators after 24 h growth and stored at 4˚C. Count the plaques on each plate and enter data in table.

Incubation temperature	Number of plaques
32˚C	
37˚C	
42˚C	

PCR of tape measure protein (TMP) gene

Using your cell phone image, analyze your results on a yes-or-no basis:

1. Did your controls work?
2. For each of the primers, note whether or not you have a DNA band. (You will do a more detailed analysis outside of lab.) Enter the data in this table.

Primer	Y or N
A1	
A2	
A3	
A4	
A5 mix	
A6	
B1	

B3	
E	
F1	
F2	
G	
K	
N	

3. Enter the data in the master table at the front of the room.